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ISWI Chromatin Remodelers Sense Nucleosome Modifications to Determine Substrate Preference

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ATP-dependent chromatin remodelers regulate access to genetic information by controlling nucleosome positions *in vivo*¹. However, the mechanism by which remodelers discriminate between different nucleosome substrates is poorly understood. Considering that many chromatin remodeling proteins possess conserved protein domains that interact with nucleosomal features², we employed a quantitative high-throughput approach, based on use of a DNA-barcoded mononucleosome library, to profile the biochemical activity of human ISWI remodelers in response to a diverse set of nucleosome modifications. We show that accessory (non-ATPase) subunits of ISWI remodelers can distinguish between differentially modified nucleosomes, directing remodeling activity toward specific nucleosome substrates based on their modification state. Unexpectedly, we discover the nucleosome acidic patch³ as necessary for maximum activity of all ISWI remodelers evaluated. We find this dependence also extends to CHD and SWI/SNF family remodelers, suggesting the acidic patch may be generally required for chromatin remodeling. Critically, we observe that remodeling activity can be regulated by modifications neighboring the acidic patch, signifying it may act as a tunable interaction hotspot for ATP-dependent chromatin remodelers and, by extension, many other chromatin effectors that engage this region of the nucleosome surface⁴⁻⁹.

Typically, individual members of a chromatin remodeling family possess the same core ATPase subunit (or homolog thereof), yet are able to perform functionally distinct roles within the nucleus². This specialization occurs through partnership with accessory protein subunits, which can offer additional protein binding partners or

sensory inputs that direct chromatin remodeling activity to specific genomic loci. The underlying molecular mechanisms that control this functional specialization, however, remain largely unclear. Many ATP-dependent chromatin remodelers possess protein domains that interact with DNA, histones, and histone post-translational modifications (PTMs)², providing the potential for these domains to act as means to interpret unique chromatin landscapes by modulating binding to nucleosomes or the DNA translocation efficiency of the ATPase. Though these effects have long been proposed, only a small number of studies have described how modifications to nucleosomes, which occur in massive complexity in vivo¹⁰, affect remodeling efficacy¹¹⁻¹⁷. To date, there has been no systematic analysis of how remodeling activity is tuned by the presence of nucleosome modifications.

Recently, we described an approach for accelerated biochemical analysis of chromatin effectors based on the use of DNA-barcoded mononucleosome libraries¹⁸. We created a new and greatly expanded version of this library (Fig. 1, Extended Data Fig. 1-3 and Supplementary Table 1), that encompassed a broad range of modifications distributed throughout the nucleosome structure, and was specifically tailored for studying chromatin remodeling using a modified restriction enzyme accessibility assay¹⁹ (Fig. 2a). We used this library to profile the remodeling behavior (i.e. nucleosome sliding) of seven recombinantly produced human ISWI remodelers; namely the SNF2h ATPase and the ACF, CHRAC, WICH, NoRC, RSF and NURF complexes (Extended Data Fig. 4). In total, this effort generated 784 remodeling rate constants from 28,224 individual kinetic measurements (Fig. 2b, Extended Data Fig. 5, Supplementary Tables 2, 3). Several built-in controls served to validate this dataset. As expected, remodeling

activity was, in all cases, negligible in the absence of ATP (Fig. 2c, d, Extended Data Fig. 5, Supplementary Table 3). Additionally, and consistent with literature precedent, nucleosomes containing modifications or mutations in the basic patch of the H4 tail were found to be poor substrates of ISWI remodelers^{15,20}, whereas nucleosomes containing the histone variant H2A.Z led to enhanced remodeling activity¹⁷ (Fig. 2e). Finally, restriction enzyme cleavage of a DNA standard present in the library that is unoccupied by a histone octamer (DNA Standard 2) was always faster than for nucleosome remodeling, ensuring its activity was not limiting in our assay.

Inspection of the kinetic data revealed that ISWI remodelers are broadly sensitive to nucleosome modifications, with both stimulatory and inhibitory effects evident for all seven enzymes tested (Figure 2b, and Supplementary Table 3). We performed principal component analysis (PCA) to identify experimental trends that contributed to variability in remodeler activity toward different nucleosome substrates. The first principal component accounted for the large majority of variance (83%) in the data (Extended Data Fig. 6a). Notably, all remodelers had very similar weight values for PC1, demonstrating that histone modifications that stimulate or impede remodeling tend to do so similarly over all the enzymes tested. This suggests remodelers share functional motifs (for example, in the common ATPase subunit) that are similarly sensitive to changes in the substrate structure, and/or certain modifications affect the stability of the nucleosome to make DNA translocation more or less difficult (Supplementary Fig. 2).

The second and third principal components reveal how the activity of the remodelers varies in response to specific nucleosome substrates (Figure 3a). Notably, PCA showed that the activity of the isolated SNF2h ATPase is distinct from all

complexes that incorporate the ATPase for DNA translocation activity, indicating the role that bound accessory subunits play in repurposing its remodeling activity in response to individual nucleosome modification states. We find that subsets of the nucleosome library drive differences in remodeler activity; the most prominent of which being the H3K14ac modification, which activates only the NoRC complex (Figure 3b, Extended Data Fig. 6b, Supplementary Table 4). Indeed, we found many individual cases where a specific nucleosome type had a broad range of effects across different remodelers analyzed (Fig. 3b), providing further evidence that the unique subunit compositions of each complex play a direct role in determining their functional specialization. We also observed that histone modifications located on more accessible regions of the nucleosome (e.g. on the flexible tails) show more variability in their effects across different remodelers as compared to those residing under the DNA, which generally elicited similar behavior across the remodelers evaluated, likely due to their ability to alter histone-DNA contacts and affect the DNA translocation efficiency of the ATPase subunit²¹ (Fig. 3c and Extended Data Fig. 7a, b).

Further analysis of the remodeling dataset provided insights into the relationship between remodeling activity and the binding preferences of histone recognition ('reader') domains present within the ISWI remodelers. This was particularly enlightening for the NoRC, WICH, and NURF complexes, all of which have reader domains within their accessory subunits whose binding preferences have been studied²²⁻²⁴. In the case of the NoRC complex, we find that the binding preferences of associated reader domains are an excellent predictor of remodeling output (Extended Data Fig. 8a). This relationship was much less pronounced for the WICH complex

(Extended Data Fig. 8b), and, in the case of the NURF complex, we observed an inverse correlation between expected binding preferences and remodeling activity (Extended Data Figs. 8c, 9a). In considering the origins for such behavior, it is worth noting that binding studies on chromatin factors typically employ isolated reader domains and/or histone-derived peptides^{25,26}. These simplified systems, while often informative, are not subject to the same structural and steric constraints present in a more physiological setting. Our data highlight the advantages of performing biochemical measurements with intact chromatin effectors and nucleosomal substrates.

Unexpectedly, we found that a nucleosome within our library in which the so-called 'acidic patch'³ had been disrupted through mutation was inefficiently remodeled by all ISWI family remodelers examined (Fig. 4a). The nucleosome acidic patch is a unique surface feature created by a cluster of acidic residues located within the core regions of histones H2A and H2B that together create a negatively charged cleft on each face of the nucleosome disc (Fig. 4a). This surface epitope has emerged as an important component of chromatin recognition⁴⁻⁹. Through further mutagenesis studies, we were able to show that ISWI remodeling activity requires a trio of amino acids in H2A (E61, D90 and E92) that are known to form a binding pocket for arginine residues within other trans-acting chromatin factors (Fig. 4b, Extended Data Fig. 9b)²⁷. Further implicating this region, remodeling of unmodified nucleosomes by the ACF complex was inhibited by addition of the latency-associated nuclear antigen (LANA) peptide from the Kaposi sarcoma-associated herpes virus, which is known to bind the acidic patch using the arginine anchor paradigm⁸ (Extended Data Fig. 9c). We also found that ATPases from the CHD family (CHD4) and SWI/SNF family (BRG1) of remodelers are sensitive

to the acidic patch, suggesting that it may, in fact, be generally required for chromatin remodeling (Fig. 4b).

The number of proteins known to bind and utilize the acidic patch in their interaction with chromatin is continually rising²⁷. It is thus intriguing to speculate that modifications in the neighborhood of the acidic patch alter the binding of ligands, perhaps in a differential manner. For example, H2A.Z provides two additional residues that extend the acidic patch and enhance ISWI remodeling activity¹⁷. Other studies hint at this possibility as well²⁸. Analysis of our dataset reveals that ISWI remodeling activity is indeed affected by a range of diverse histone PTMs located near the acidic patch (Fig. 4a). Notably, both stimulation and inhibition of nucleosome sliding activity was observed in response to the PTMs (Fig. 4a, Extended Data 9d). The activity profile of each remodeler was similar across this substrate set, suggesting that a common sensing mechanism was operational across the ISWI remodeler family. We anticipate such a mechanism is mediated by the ATPase subunit, since it is the only shared component of the complexes. Consistent with this assignment, the isolated SNF2h ATPase subunit was also found to require an intact acidic patch for activity (Fig. 4a). We imagine the acidic patch may act through stabilizing a SNF2h-nucleosome interaction, or, in considering known modes of ISWI regulation by nucleosomal epitopes²⁹, we can also envision possible allosteric mechanisms.

Based on the above, we used our nucleosome library platform to investigate the possibility that certain PTMs or histone variants may act as 'selectivity filters' for the acidic patch, granting access to subsets of chromatin effectors. In addition to ISWI remodelers, we observed this to be the case for CHD4, RCC1, and Sir3, all factors that

we demonstrate, or have previously been demonstrated^{5,6}, to engage the acidic patch (Fig. 4c, Extended Data Fig. 10, Supplementary Table 3, and Supplementary Table 7). We note that compared to PTMs within histone N-terminal tails, which have been extensively studied with respect to chromatin regulation, those residing within the core of the histone octamer are much more enigmatic²¹. We imagine that the acidic patch is able to act as a compositionally dynamic and regulatable interaction hotspot for chromatin effector proteins (Fig. 4d). By extension, perturbation of this region of the nucleosome either through aberrant PTM installation or mutation could lead to dysregulation of epigenetic processes. Indeed, we note that a mutation in the acidic patch (H2AE56Q) has recently been implicated in human uterine and ovarian carcinomas³⁰.

By performing the first systematic analysis of the effects of nucleosome modifications on chromatin remodeling activity, we have generated a dataset that exists as a resource that should drive the design of future biochemical and cell-based studies geared towards further understanding ISWI regulation. We also discover the nucleosome acidic patch is generally required for ISWI remodeling as well as for the activity of chromatin remodeling enzymes spanning multiple remodeling families. Additionally, our data lead us to propose the acidic patch is subject to dynamic regulation by nucleosome modifications. Lastly, the library developed herein will have broad utility in chromatin biochemistry, by allowing causal relationships between histone modifications and enzymatic outputs to be explored in an unbiased and quantitative manner.

Author Contributions

G. P. D. purified all remodelers, performed all remodeling and nucleosome library experiments, and processed all sequencing data; G. P. D prepared the nucleosome library with contributions from G. P. L., M. M. M., U. T. T. N., T. P., Z. Z. B., F. W., J. B., R. P., and S. B. P.; G. P. D and G. P. L. purified RCC1 and Sir3 constructs for nucleosome library binding experiments; G. P. L. performed the qPCR analysis; G. P. D. and K. L. D. synthesized and purified the LANA peptides; J. D. B. performed the PCA analysis; C. D. A. oversaw the preparation of crotonylated histones; G. P. D, J. D. B., and T. W. M. analyzed the data; G. P. D., M. M. M., and T. W. M. conceived the project; T. W. M. supervised the study; G. P. D. and T. W. M. wrote the manuscript.

Author Information

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FIGURE LEGENDS

Figure 1 | A Diverse Library of Modified Nucleosomes.

Diagram depicting all histone modifications, mutants, and variants present in the 115-member nucleosome library used in this study. Residues modified or mutated were mapped on to the nucleosome (PDB: 1KX5) in black using UCSF chimera. H2A (light yellow), H2B (light red), H3 (light blue), and H4 (light green) modification and mutation locations are indicated by boxes and lines. For clarity, connections are only shown to a single copy of each histone protein.

Figure 2 | A High-throughput Nucleosome Remodeling Assay for ISWI Family Chromatin Remodelers.

a, Schematic of the restriction enzyme accessibility assay used with the DNA-barcoded library. Individual remodeling rates are calculated from unique DNA sequencing reads. **b**, Heat-map displaying ISWI remodeling data against the nucleosome library. Rows were sorted based on values for SNF2h (low to high). k_{MN} = nucleosome remodeling rate. $k_{unmod.}$ = unmodified nucleosome remodeling rate. Values were capped at -4 and 4 for display purposes. **c**, Example decay curves depicting individual rates (k_{MN}) as in **b**. **d**, Rank-ordered remodeling rates for the ACF complex (k_{MN}) against the library. Dashed red line = $k_{unmod.}$ **e**, Relative remodeling rates as in **b** for select library members. All data are represented as the mean of experimental replicates (n=3). Error bars represent \pm s.e.m. All histones are unmodified unless otherwise specified.

Figure 3 | Specialization of ISWI Remodelers Toward Diverse Nucleosome Modifications.

a, Principal component analysis of library remodeling data. Nucleosomes = light blue; PC weight values for remodelers = orange. Weights are scaled by a factor of 2 for visibility. **b**, ISWI remodeling rates for select nucleosome substrates in the library. k_{MN} =

nucleosome remodeling rate. $k_{\text{unmod.}}$ = unmodified nucleosome remodeling rate. Values were capped at -4 and 4 for display purposes. All histones are unmodified unless otherwise specified. **c**, Single-site modifications mapped onto the nucleosome (PDB: 1KX5) and colored based on whether they had consistently positive (green), consistently negative (red), or variable (purple) effects on nucleosome remodeling activity across all ISWI remodelers analyzed.

Figure 4 | The Nucleosome Acidic Patch is Crucial for Remodeling and Regulatable by Histone PTMs.

a, Coulombic surface rendering of the nucleosome (PDB: 1KX5, far right). (Far left) Effect of acidic patch modifications and mutations on ISWI remodeling activity. k_{MN} = nucleosome remodeling rate. $k_{\text{unmod.}}$ = unmodified nucleosome remodeling rate. Mutations and PTM locations are individually numbered on the nucleosome structure (middle panel) with decimals indicating multiple changes per nucleosome (7.1 = H2AE56A 7.2 = H2BE113A, 7.3 = H2AE61A, 7.4 = H2AE64A, 7.5 = H2AD90A, 7.6 = H2AE92A, 7.7 = H2BE105A, 7.8 = H2AE91A; 2.1 = N94D (H2A→H2A.Z), 2.2 = K95S (H2A→H2A.Z). Values were capped at -4 and 4 for display purposes. **b**, E61A, D90A, and E92A mutations in H2A reduce remodeling activity of the ACF complex, CHD4, and BRG1 as read out by a gel-based restriction enzyme accessibility assay (corresponding example replicates shown on right). **c**, Acidic patch modifications differentially affect remodeling activity and binding of chromatin factors relative to unmodified nucleosomes. For complete data sets see Extended Data Fig. 10, Supplementary Table 3 and Supplementary Table 7. **d**, Model for how histone modifications (yellow triangle, red square) proximal to the acidic patch (pink) might differentially regulate the binding and hence function of chromatin factors. For **a** and **b** all histones are unmodified unless otherwise specified. For **b** and **c** data are represented as the mean of experimental replicates (n=3). Error bars represent \pm s.e.m. For gel source data, see Supplementary Figure 1.

METHODS

Analytical methods

Analytical reversed-phase HPLC (RP-HPLC) was performed on an Agilent 1100 series instrument employing a Vydac C18 column (5 μm , 4 \times 150 mm) at a flow rate of 1 mL/min. Semi-preparative scale purifications were performed on an Agilent 1100 series instrument employing a Vydac C18 semipreparative column (12 μm , 10 mm \times 250 mm) at a flow rate of 4 mL/min. Preparative scale purifications were conducted on a Waters DeltaPrep 4000 system equipped with a Waters 486 tunable detector (Waters). A Vydac C18 preparative column (15–20 μm , 20 \times 250 mm) was used at a flow rate of 20 mL/min. 0.1% TFA in water (HPLC solvent A) and 90% acetonitrile, 0.1% TFA in water (HPLC solvent B) were used as the mobile phases in all RP-HPLC analyses and purifications. ESI-MS analysis was conducted on a MicrOTOF-Q II ESI-Qq-TOF mass spectrometer (Bruker Daltonics).

Production and purification of RCC1 and Sir3 constructs

Full-length RCC1 (Uniprot ID: P18754) with an N-terminal 6xHis-SUMO tag and a C-terminal GST tag and the BAH domain of *S. cerevisiae* Sir3⁶ with a C-terminal FLAG-6xHis tag were subcloned into bacterial expression plasmids (pET, Novagen) for protein production in *E. coli*. In brief, BL21 Rosetta™ (DE3) cells were transfected with expression plasmids and grown in LB medium at 37 °C until reaching an OD₆₀₀ of 0.6. For the 6xHis-SUMO-RCC1-GST construct, protein expression was induced by the addition of 0.6 mM IPTG overnight at 18 °C. For the Sir3-BAH-FLAG-6xHis construct, protein expression was induced by the addition of 0.6 mM IPTG for 1h at 37 °C. Cells were harvested by centrifugation at 4,000 x g for 10 minutes at 4 °C, and cell pellets were washed twice with 10 mL of cold PBS per L of culture. Cells were lysed in 10 mL of lysis buffer (40 mM Tris, 1 M NaCl, 5 mM β-mercaptoethanol, 10% glycerol, pH 7.7 at 4 °C) per L of culture by passage through an EmulsiFlex-C3 homogenizer (Avestin). Each protein was initially purified by Ni-NTA affinity chromatography. The 6xHis-SUMO tag was cleaved from the RCC1 construct by Ulp1 protease, and removed by reverse Ni-NTA affinity chromatography. The RCC1-GST and Sir3-BAH-FLAG-6xHis constructs were further purified over Superdex 200 10/300 GL and Superdex 75 10/300 GL gel filtration columns (GE Healthcare) equilibrated with gel filtration buffer (50 mM Tris, 100 mM NaCl, 2 mM tris(2-carboxyethyl)phosphine, 10% glycerol), respectively, using an AKTA FPLC system (GE Healthcare) equipped with a P-920 pump and UPC-900 monitor prior to use in subsequent experiments.

Production and purification of recombinant histones

Unmodified recombinant human histones (H2A, Uniprot ID: Q6FI13; H2B, Uniprot ID: O60814; H3C96A, C110A, Uniprot ID: P68431; H4, Uniprot ID: P62805) histone variants, and histone mutants were produced in and purified from *E. coli*. In brief, BL21 Rosetta™ (DE3) cells were transfected with histone expression plasmids (pET, Novagen) and grown in LB medium at 37 °C until reaching an OD₆₀₀ of 0.6. Protein expression was induced by the addition of 0.6 mM IPTG for 2-3 hours at 37 °C. Cells were harvested by centrifugation at 4,000 x g for 10 minutes at 4 °C, and cell pellets were washed twice with 10 mL of cold PBS per L of culture. Cell pellets were resuspended in 10 mL of cold lysis buffer (50 mM Tris, 100 mM NaCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 7.6 at 4 °C) with cOmplete™, EDTA-free Protease Inhibitor Cocktail (Sigma-Aldrich) per L of culture and homogenized by passage several times through an 18-gauge needle. Cells were lysed via sonication and the resulting suspension was centrifuged at 30,000 x g for 30 minutes at 4 °C. The inclusion body pellet was washed twice with cold lysis buffer containing 1% Triton-X 100 and once without detergent. Inclusion body pellets were resuspended in 10 mL of inclusion body resuspension buffer (6 M guanidine hydrochloride, 20 mM Tris, 1 mM EDTA, 100 mM NaCl, 1 mM DTT, pH 7.5 at 4 °C) per L of culture, and nutated at 4 °C for 2 hours. Resuspensions were then centrifuged at 30,000 x g for 30 minutes at 4 °C, and the supernatants were transferred to 3.5 kDa MW cutoff dialysis tubing and dialyzed overnight in 2 L of low salt urea buffer (7 M urea, 10 mM Tris, 1 mM EDTA, 100 mM NaCl, 1 mM DTT, pH 7.5 at 4 °C). Resuspensions were dialyzed an additional 2 hours prior to loading onto a HiTrap SP HP 5 mL column (GE Healthcare) equilibrated with

low salt urea buffer (filtered and degassed). The histones were purified using a gradient over 20 column volumes from low salt urea buffer to high salt urea buffer (7 M urea, 10 mM Tris, 1 mM EDTA, 1 M NaCl, 1 mM DTT, pH 7.5 at 4 °C) on an AKTA FPLC system from GE Healthcare equipped with a P-920 pump and UPC-900 monitor. Histones were further purified using preparative C-18 RP-HPLC. Purified histones were analyzed by analytical HPLC and ESI-MS (Extended Data Fig. 1).

Production of modified histones by protein semi-synthesis

H2AK119ub, H2BK120ub, H3K4me3, H3K9ac, H3K9me3, H3K14ac, H3K18ac, H3K18acK23ac, H3K23ac, H3K27ac, H3K27me3, H3KpolyAc, H4K5ac, H4K8ac, H4K12ac, H4K16ac, H4K20ac, and H4KpolyAc histones were prepared by semisynthesis as described previously¹⁸. The semisynthesis of the H3R42me2a histone was conducted as described previously³¹.

Preparation of H2AK5ac, H2AK9ac, H2AK13ac, H2AK15ac, and H2AKpolyAc histones

Proteins were assembled from two pieces: A recombinant fragment was generated encompassing residues 21-129 of histone H2A with an A21C mutation fused to an N-terminal 6xHis-SUMO tag. This sequence was inserted into a pET expression plasmid and confirmed by gene sequencing. BL21 Rosetta™ (DE3) cells were transfected with the plasmid and grown in LB medium at 37 °C until reaching an OD₆₀₀ of 0.6. Protein expression was induced by the addition of 0.6 mM IPTG for 2-3 hours. Cells were harvested, lysed, and inclusion bodies were prepared identically as for recombinant histones in this study. The 6xHis-SUMO-H2A(21-129)A21C protein was purified by Ni-NTA affinity chromatography. The sample was dialyzed for 2 hours in 2 M urea, 50 mM Tris, 200 mM NaCl, 1 mM DTT, pH 7.4 at 4 °C before being moved to an otherwise identical buffer containing 1.5 M urea. Ulp1 protease was added to cleave off the 6xHis-SUMO and the sample was dialyzed overnight at 4 °C. The resulting H2A(21-129)A21C fragment was purified using preparative C-18 RP-HPLC. The final product was characterized by ESI-MS and analytical C-18 RP-HPLC.

H2AK5ac, H2AK9ac, H2AK13ac, H2AK15ac, and H2AKpolyAc peptides corresponding to residues 1-20 with acetylated lysine residues at the indicated positions were synthesized as follows: approximately 500-750 mg of Trityl-OH resin (ChemMatrix) resuspended with 10 mL of dichloromethane (DCM) and chlorinated by reaction with 200 µL of SOCl₂ on a shaker overnight. The resin was washed thoroughly with DCM and dimethylformamide (DMF) followed by an additional wash with 5% (v/v) *N,N*-diisopropylethylamine (DIEA). 500 mg of chlorinated resin was combined with 4-fold excess (relative to resin loading) of hydrazine monohydrate in 2 mL DMF along with an 8-fold excess of DIEA. The reaction was stirred on ice for 1 hour and subsequently washed with DMF prior to repeating the reaction with hydrazine/DIEA in DMF to ensure quantitative loading. To ensure all reactive sites on the resin had been consumed, after 1 hour, 500 µL of methanol was added to the second hydrazine reaction and allowed to stir for an additional 30 minutes.

The peptides were synthesized on a CEM Discover Microwave Peptide Synthesizer using Fmoc chemistry. Fmoc-acetyllysine was used to incorporate

acetyllysine residues at the appropriate positions. For a 0.25 mmol-scale reaction the resin was first swelled in DMF. In between, the resin was washed with DMF and DCM. Each amino acid was dissolved in DMF (200 mM) and introduced to the resin after the deprotection step. DIEA (2 M) in *N*-methylpyrrolidone (NMP) and HOBt (500 mM)/HBTU (500 mM) in DMF were added along with the amino acid to perform each coupling (twice). The resin was then removed from the synthesizer without deprotecting the final Fmoc group for stability in storage. The N-terminus of each peptide was manually deprotected with 20% piperidine and acetylated with acetic anhydride. Peptides were cleaved from the resin as C-terminal acyl hydrazides in 95% TFA, 2.5% triisopropylsilane (TIS), and 2.5% water and purified by preparative C-18 RP-HPLC. The final products were characterized by ESI-MS and analytical C-18 RP-HPLC.

Full-length histones were prepared by traceless native chemical ligation of acetylated peptides and the recombinant fragment as previously described¹⁸ and purified by semi-preparative C-18 RP-HPLC. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of the H2AK118ac histone

A recombinant fragment corresponding to residues 1-112 of H2A was fused to a fused Npu DnaE intein with a C-terminal 6xHis tag. This protein was produced in *E. coli* and purified by Ni-NTA affinity chromatography. An α -thioester was installed in the histone fragment by thiolysis (via 2-mercaptoethanesulfonate) of the corresponding intein fusion. An H2AK118ac peptide (113-129, A113C) was synthesized as previously described in the methods section 'Preparation of H2AK5ac, H2AK9ac, H2AK13ac, H2AK15ac, and H2AKpolyAc histones', with minor modifications. The peptide was synthesized on Wang resin that was purchased pre-charged with the C-terminal amino acid (Boc-protected lysine). Acetylation of the N-terminal amine with acetic anhydride was omitted after final Fmoc deprotection, and the peptides were cleaved (95% TFA, 2.5% triisopropylsilane (TIS), and 2.5%) as C-terminal carboxylates. Full-length histones were prepared by traceless native chemical ligation of acetylated peptide and the recombinant fragment as previously described¹⁸ and subsequently purified by semi-preparative C-18 RP-HPLC. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of H2BK5ac, H2BK11ac, H2BK12ac, H2BK15ac, H2BK16ac, and H2BK20ac histones

Proteins were assembled from two pieces: A recombinant fragment was generated encompassing residues 21-125 of histone H2B with an A21C mutation fused to an N-terminal 6xHis-SUMO tag. This sequence was inserted into a pET expression plasmid and confirmed by gene sequencing. The recombinant H2B(21-125)A21C fragment was produced and purified analogous to that described for the H2A(21-129)A21C fragment in the methods section 'Preparation of H2AK5ac, H2AK9ac, H2AK13ac, H2AK15ac, and H2AKpolyAc histones'. The final product was characterized by ESI-MS and analytical C-18 RP-HPLC.

H2BK5ac, H2BK11ac, H2BK12ac, H2BK15ac, H2BK16ac, and H2BK20ac peptides corresponding to residues 1-20 with acetylated lysine residues at the indicated positions were synthesized analogous to that described in the methods section 'Preparation of H2AK5ac, H2AK9ac, H2AK13ac, H2AK15ac, and H2AKpolyAc histones', omitting acetylation of the N-terminal amine with acetic anhydride. Full-length histones were prepared by traceless native chemical ligation of acetylated peptides and the recombinant fragment as previously described¹⁸ and were purified by semi-preparative C-18 RP-HPLC. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of H2BK108ac, H2BK116ac, H2BK120ac, H2BK125ac histones

H2BK108ac, H2BK116ac, H2BK120ac, and H2BK125ac histones were produced in a similar manner to H2AK118ac as described in the methods section 'Preparation of the H2AK118ac histone'. A recombinant fragment corresponding to residues 1-106 of H2B containing an α -thioester was prepared via thiolysis of fused Npu DnaE intein fusion for ligation to H2BK108ac(107-125, A107C) and H2BK116ac(107-125, A107C) peptides. Additionally, a recombinant fragment corresponding to residues 1-116 of H2B containing an α -thioester was prepared via thiolysis of a fused Npu DnaE intein fusion for ligation to H2BK120ac(117-125, A107C) and H2BK125ac(117-125, A117C) peptides. Full-length histones were prepared by traceless native chemical ligation of acetylated peptides and the recombinant fragment as previously described¹⁸ and were subsequently purified by semi-preparative C-18 RP-HPLC. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of phosphorylated H3 histones

The H3H41ph histone was prepared using a previously described 3-piece traceless ligation strategy³¹ with minor modifications. Notably, the middle peptide fragment (residues 29-46, A29C) was synthesized with inclusion of Fmoc-Tyr(PO(OBzl)OH)-OH, which was used to introduce a phosphotyrosine residue at position 41 of H3. The H3S10ph histone was prepared using a 2-piece traceless ligation strategy analogous to that described previously for ligation of synthetic peptide fragments corresponding to residues 1-14 of H3 to a recombinant H3 fragment corresponding to residues 15-135 (A15C, C96A, C110A)¹⁸. The N-terminal peptide fragment (residues 1-14) was synthesized with inclusion of Fmoc-Ser(PO(OBzl)OH)-OH to introduce a phosphoserine residue at position 10 of H3. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of H3K4me1 and H3K4me2 histones

H3K4me1 and H3K4me2 histones were prepared using a 2-piece traceless ligation strategy analogous to that described previously for native chemical ligation of synthetic peptide fragments corresponding to residues 1-28 of H3 to a recombinant H3 fragment corresponding to residues 29-135 (A29C, C96A, C110A)¹⁸. The N-terminal peptide fragments (residues 1-28) were synthesized using Fmoc chemistry as

previously described utilizing an on-resin alkylation strategy to generate mono and dimethylated lysine residues at position 4 of H3^{32,33}. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of H3R2 methylated histones

H3R2me and H3R2me2a histones were prepared using a 2-piece traceless ligation strategy analogous to that described previously for ligation of synthetic peptide fragments corresponding to residues 1-14 of H3 to a recombinant H3 fragment corresponding to residues 15-135 (A15C, C96A, C110A)¹⁸. The N-terminal peptide fragment (residues 1-14) was synthesized with inclusion of either Fmoc-Arg(Me,Pbf)-OH or Fmoc-ADMA(Pbf)-OH to introduce either a monomethyl or asymmetric dimethyl arginine residue at position 2 of H3. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of the H3K36me3 histone

The H3K36me3 histone was prepared with a 3-piece ligation strategy analogous to that previously described³¹ with minor modifications. Notably, the full-length sequence corresponded to H3 and contained C96A, C110A mutations, and the middle peptide fragment (residues 29-46, A29C) was synthesized with inclusion of Fmoc-Lys(Me₃)-OH, which was used to introduce a trimethyllysine residue at position 36 of H3. The final product was characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of H4R3 methylated histones

Synthetic H4 peptides were assembled as alkyl-thioesters (TAMPAL) on solid phase. Up to residue 4, amino acids were incorporated using Boc-protected amino acids and *in situ* neutralization protocols. Side-chain protected amino acids (H4(1-14)R3Me and R3Me2s) were coupled using standard HBTU/DIPEA procedures; subsequent couplings were carried out using Fmoc amino acids, thus maintaining acid sensitive side-chain protecting groups (e.g. Boc or Pbf). To reduce Fmoc-deprotection cocktail exposure time, the terminal residue could be coupled with Boc-protection. Final Fmoc deprotection was carried out in 1-methylpyrrolidine (25 % v/v), hexamethylene imine (2 % v/v), HOBt (2 % w/v), in NMP-DMSO (1:1); (2) DBU (1 % v/v), HOBt (1 % w/v) in DMF.

The H4(1-14)R3Me2a thioester peptide was synthesized, using resin from above, that was split prior to coupling methyl arginine residues. Following Boc-deprotection, neutralization of the TFA salt (5 % (v/v) DIPEA-DMF), and thorough washing with DMF, the resin was washed with 5 % (w/v) HOBt-DMF, ensuring a slightly acidic environment necessary to maintain arginine side-chain protonation. Subsequent base-free activation of Fmoc-ADMA, in the presence of peptidyl-resin, was achieved using 5 eq of Fmoc-ADMA-OH, 5 eq of *N,N*-diisopropylcarbodiimide (DIC) and 5.5 eq of HOBt in 10 % DMF-DCM, with excess HOBt used to maintain side-chain protonation. Fmoc-deprotection was subsequently mediated using 1-methylpyrrolidine (25 % v/v),

hexamethylene imine (2 % v/v), HOBt (2 % w/v), in NMP-DMSO (1:1); (2) DBU (1 % v/v), HOBt (1 % w/v) in DMF, and the described coupling process repeated for subsequent amino acids, with neutralization omitted following Fmoc-deprotection.

Peptides were cleaved from the resin using liquid HF. HF cleavage (10mL HF/g resin) was performed for 1.5 h at 0-4 °C with 5 % (v/v) p-cresol as a scavenger. Following HF cleavage, the HF was removed under reduced pressure, peptides were precipitated in ice-cold ether, filtered, dissolved in 40% (v/v) aqueous MeCN containing 0.1% (v/v) TFA, and lyophilized. Peptides were purified by preparative C-18 RP-HPLC and final products were characterized by analytical C-18 RP-HPLC and ESI-MS.

To generate the remaining recombinant portion of H4 suitable for native chemical ligation, the H4(1-14) tail was fused to the hexahistidine-tagged, thrombin cleavable truncated H4 protein bearing an A15C mutation – insertion of the His-tag and thrombin cleavage sequence into the H4 protein between residues 14 and 15 was found to improve expression levels. This approach yielded protein as inclusion bodies, which after Ni²⁺-affinity chromatography, was subjected to on-column cleavage with thrombin. By incorporating a mutated thrombin cleavage site (LVPRC), the desired N-terminal cysteine containing H4 fragment could be obtained by extracting the cleaved protein from the column using 8 M urea. The truncated histones were then further purified by preparative C-18 RP-HPLC.

Native chemical ligation was performed in 0.1 M sodium phosphate pH 7.5, 6 M guanidine-HCl, 0.1 M NaCl, 60 mM TCEP, 30 mM 4-mercaptophenylacetic acid (MPAA) at 37 °C. Reactions were performed at approximately 3 mM peptide concentration in siliconized 1.5 mL microcentrifuge tubes. Significantly lower yields were obtained when standard polypropylene tubes were used. Ligation products were purified, desulfurized, and purified analogous to that previously described¹⁸. Final protein products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of H4K77ac and H4K79ac histones

H4K77ac and H4K79ac histones were produced similarly as described in the methods section 'Preparation of the H2AK118ac histone'. A recombinant fragment corresponding to residues 1-75 of H4 containing an α -thioester was prepared via thiolysis of a fused Npu DnaE intein fusion for ligation to H4K77ac(76-102, A76C) and H4K79ac(76-102, A76C) peptides. Full-length histones were prepared by traceless native chemical ligation of acetylated peptides and the recombinant fragment analogous to that previously described¹⁸ and were subsequently purified by semi-preparative C-18 RP-HPLC. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Preparation of H2BS112GlcNAc

Fmoc-Ser(β -D-GlcNAc(Ac)₃)-OH was synthesized as previously described (using aglycone Fmoc-Ser(OH)-OBn and glycosyl donor GlcNTroc(Ac)₃-Br)³⁴ for use in solid-phase peptide synthesis. Standard Fmoc solid-phase peptide synthesis was used to access the glycopeptide H2B(107-125, S112GlcNAc, A107C). After successful peptide assembly, the peracetylated glycopeptide was deprotected on resin by hydrazinolysis³⁴,

cleaved from the resin, and purified by preparative C18 RP-HPLC. H2B(1-106) α -thioester was obtained by expression and thiolysis of a recombinant intein fusion fragment of H2B, H2B(1-106)GyrA-His₆. The final glycosylated H2BS112GlcNAc histone was prepared by traceless native chemical ligation of the recombinant H2B fragment and the glycopeptide analogous to that previously described¹⁸ and was subsequently purified by semi-preparative RP-HPLC. The final product was characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Production and purification of modified histones by amber suppression

All crotonylated histones were produced using amber suppression in *E. coli* as previously described³⁵.

Preparation of H3K4ac, H3K36ac, H3K37ac, H3K56ac, H3K64ac, H3K79ac, H3K115ac, H3K122ac, and H4K91ac histones

H3K4ac, H3K36ac, H3K37ac, H3K56ac, H3K64ac, H3K79ac, H3K115ac, H3K122ac histones were prepared by fusing a fused Npu DnaE intein to the C-terminus of H3 (H3C96A, C110A) or H4 and produced using amber suppression in *E. coli* as previously described^{12,36}. Following purification, all proteins were characterized by analytical C-18 RP-HPLC and ESI-MS (Extended Data Fig. 1).

Synthesis of LANA peptides

Two LANA peptides⁸ corresponding to residues 2-22 with a norleucine at position 6 substituted for methionine were prepared. The first contained the otherwise native sequence, and the second had residues 8-10 (LRS) mutated to alanine, which is known to hinder nucleosome binding⁸. Peptides were synthesized on a CEM Discover Microwave Peptide Synthesizer using Fmoc chemistry and a ChemMatrix Rink amide resin (0.47 mmol/g). Following chain assembly, peptides were cleaved from the resin using 95% TFA, 2.5% TIS, and 2.5% water and purified by C-18 RP-HPLC. Final products were characterized by analytical C-18 RP-HPLC and ESI-MS (Supplementary Fig. 3).

Production of chromatin remodelers in Sf9 cells

All chromatin remodelers used in this study were produced in Sf9 cells using a baculovirus expression vector system. The SNF2h (Uniprot ID: O60264), ACF1 (Uniprot ID: Q9NRL2), WSTF (Uniprot ID: Q9UIG0), CHRAC-15 (Uniprot ID: Q9NRG0), and CHRAC-17 (Uniprot ID: Q9NRF9) coding sequences were a gift from R. Kingston (Harvard University). The RSF1 (Uniprot ID: Q96T23) coding sequence was a gift from D. Reinberg (New York University). The BPTF (Uniprot ID: Q12830, Isoform 4) coding sequence was a gift from C. D. Allis (Rockefeller University). The SNFL, TIP5 (Uniprot ID: Q9UIF9, Isoform 1), CHD4 (Uniprot ID: Q14839), and RbAp46 (Uniprot ID: Q16576) coding sequences were purchased from Open Biosystems. The SNF2L coding sequence was purchased as an inactive splice variant³⁷ with an insertion that was

deleted using standard molecular cloning procedures to yield the active variant (Uniprot ID: P28370, Isoform 2). pFastBac1-Flag-BRG1 was a gift from Robert Kingston (Addgene plasmid # 1957). All coding sequences were confirmed by Sanger sequencing. All coding sequences were subcloned into vectors compatible with bacmid generation by standard restriction enzyme cloning methods or Gibson Assembly® (NEB) with FLAG affinity tags introduced suitable for purification of all desired proteins and protein complexes (flag = N- or C-terminal FLAG affinity tag): pFastBac1-SNF2h-flag, pACEBac1-SNF2h, pACEBac1-ACF1-flag, pACEBac1-WSTF-flag, pACEBac1-CHRAc-15, pACEBac1-CHRAc-17, pACEBac1-TIP5-flag, pACEBac1-flag-RSF1, pFastBac1-flag-BPTF, pACEBac1-RbAp46, pACEBac1-SNF2L, and pACEBac1-CHD4-flag. For bacmid generation, SNF2h, ACF1-flag, WSTF-flag, CHRAc-15, CHRAc-17, TIP5-flag, flag-RSF1, CHD4-flag constructs were transfected in to DH10MultiBacTurbo™ *E. coli* competent cells, and bacmids were produced per manufacturer's instructions (Geneva Biotech). SNF2h-flag, flag-BPTF, SNF2L, RbAp46, and flag-BRG1 constructs were transfected into DH10bac *E. coli* competent cells, and bacmids were produced per manufacturer's instructions (Bac-to-Bac® Baculovirus Expression System, ThermoFisher Scientific). To generate virus for protein production, bacmid transfection into Sf9 cells was carried out in 6-well plates. All transfection, viral amplification, and infection steps were performed in a sterile hood. Typically, 1-10 µg of bacmid was transfected into 1×10^6 attached Sf9 cells according to manufacturer's instructions (Bac-to-Bac® Baculovirus Expression System, ThermoFisher Scientific). After transfection, cells were overlaid with 2 mL fresh medium (Sf-900™III SFM, Thermo Fisher Scientific) and incubated at 27 °C for 72 hours in the dark. The supernatant was collected for virus amplification and cleared by centrifugation to generate the P1 virus. 2% FBS (v/v) was added. Between uses, all viral stocks were stored at 4 °C in the dark. Subsequent steps were carried out in medium with penicillin/streptomycin. To generate the P2 virus, 800 µL P1 virus was added to 20 mL of Sf9 cells in a sterile flask at 2×10^6 cells/mL. Cells were grown at 27 °C in suspension culture in the dark until they reached 40% viability as monitored by Trypan Blue staining. The culture supernatant was then collected and cleared by centrifugation, and 2% FBS (v/v) was added. To generate the P3 virus (used for protein production), 200 µL P2 virus was added to 50 mL of Sf9 cells in a sterile flask at 2×10^6 cells/mL. Cells were grown at 27 °C in suspension culture in the dark until they reached 40% viability as monitored by Trypan Blue staining. The culture supernatant was then collected and cleared by centrifugation, and 2% FBS (v/v) was added. During virus amplification Sf9 cell density was kept around 2×10^6 cells/mL, diluting if needed, until growth arrested and viability dropped. Aside from the monomeric SNF2h (SNF2h-flag), CHD4 (CHD4-flag), and BRG1 (flag-BRG1) ATPases, individual viruses were directly combined with Sf9 cell cultures to generate the ACF (ACF1-flag, SNF2h), CHRAc (ACF1-flag, SNF2h, CHRAc-15, CHRAc-17), WICH (WSTF-flag, SNF2h), NoRC (TIP5-flag, SNF2h), RSF (flag-RSF1, SNF2h), and NURF (flag-BPTF, SNF2L, RbAp46) complexes. Production of CHD4 and the ACF, CHRAc, WICH, NoRC, and RSF complexes was carried out by adding a 1:1000 dilution of each P3 virus to Sf9 suspension cultures at 2×10^6 cells/mL. Cells were harvested by centrifugation after 72 hours at 27 °C in the dark. Production of SNF2h, BRG1, and the NURF complex was carried out by adding a 1:100 dilution of P3 virus to Sf9 suspension cultures at 2×10^6

cells/mL. Cells were harvested by centrifugation after 48 hours at 27 °C in the dark. All proteins and protein complexes were purified by first washing harvested cell pellets with 10 mL of cold TBS (50 mM Tris, 150 mM NaCl, pH 7.5 at 4 °C) per 50 mL of culture. Washed pellets were resuspended in 400 μ L of cold nuclear extraction buffer A (buffer A; 10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA) with 1 mM DTT, cOmplete™, EDTA-free Protease Inhibitor Cocktail (Sigma-Aldrich), and 0.5 mM PMSF per 10 mL of suspension culture and swelled on ice for 15 minutes. 25 μ L of 10% IGEPAL CA-630 per 400 μ L of buffer A were then added, and the cells were vortexed briefly for lysis. Nuclei were spun down at 4 °C for 30 seconds at 17,000 x g and the supernatant was removed. The nuclei were washed once with 400 μ L of cold buffer A (with 1 mM DTT, cOmplete™, EDTA-free Protease Inhibitor Cocktail, and 0.5 mM PMSF) per 10 mL of suspension culture. The resulting nuclear pellet was resuspended in 50 μ L of cold nuclear extraction buffer B (buffer B, 20 mM HEPES, pH 7.9, 400 mM KCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol) with 1 mM DTT, cOmplete™, EDTA-free Protease Inhibitor Cocktail, and 0.5 mM PMSF per 10 mL of suspension culture. Nuclear pellets were then incubated with end-over-end rotation for 15 minutes at 4 °C. Nuclear debris was then spun down at 4 °C for 10 minutes at 17,000 x g. The supernatant (nuclear extract) was removed for FLAG affinity purification of remodelers. 1.5 μ L (3 μ L of 50% slurry) of ANTI-FLAG® M2 beads (Sigma-Aldrich) per mL of suspension culture were used for purification. First, beads were washed twice with 10 bead volumes of BC-100 buffer (20 mM HEPES, 100 mM KCl, 0.2 mM EDTA, 10% glycerol, 1 mM DTT, 0.2 mM PMSF, pH 7.9). The nuclear extract was diluted 2-fold with BC-0 buffer (BC-100 buffer without KCl) and centrifuged at $\geq 4,000$ x g for 10 minutes at 4 °C. The supernatant was removed from any precipitation, added directly to the washed ANTI-FLAG® M2 beads, and incubated for 1 hour at 4 °C with end-over-end rotation. The beads were centrifuged for 5 minutes at 1000 x g and 4 °C, and the supernatant was removed. The beads were washed once with 10 bead volumes of BC-100 buffer, once with 10 bead volumes of BC-300 buffer (BC-100 buffer with 300 mM KCl), and once with 10 beads volumes of BC-100 buffer. Bound protein was eluted by incubation with one bead volume of 0.25 mg/mL FLAG peptide (Sigma-Aldrich) in BC-100 buffer for 20 minutes at 4 °C. This process was repeated twice. Pure elutions were combined and concentrated using appropriate MW cut-off Vivaspin 500 centrifugal filter units (Vivaproducts) if needed. Concentrations of remodelers were determined using BSA standards and SDS-PAGE with Coomassie blue staining referencing the intensity of the ATPase subunit for each complex. Following purification, remodelers were aliquoted, flash-frozen in liquid nitrogen and stored at -80 °C until use. Purity of ISWI family remodelers (Extended Data Fig. 4a) as well as BRG1 and CHD4 (Supplementary Fig. 4a) was assessed by SDS-PAGE analysis and Coomassie blue staining. Additionally, ATP-dependent nucleosome remodeling activity on unmodified nucleosomes was verified for ISWI family members (Extended Data Fig. 4b) as well as BRG1 and CHD4 (Supplementary Fig. 4b) using a restriction enzyme accessibility assay. All assays were performed in 50 μ L reactions with 10 nM unmodified nucleosomes with under conditions described in methods section 'Nucleosome remodeling rate analysis for validation experiments on single nucleosomes'. Time points were taken at 1 minute and 60 minutes, quenched, and deproteinized as described in the methods section 'Remodeling assays using the nucleosome library'. Samples were

directly run on a 5% polyacrylamide gel (0.5x TBE, 200 V, 40 minutes). Staining was performed with SYBR® Safe DNA gel stain, and gels were imaged on a Typhoon scanner (GE Healthcare). Each remodeler was analyzed in the presence and absence of 2 mM ATP. In all cases, the appearance of a lower band at 60 minutes that is dependent on the presence of ATP (indicative of remodeling) is visible. Concentrations of remodelers used: SNF2h: 200 nM, ACF: 2 nM, CHRAC: 20 nM, WICH: 50 nM, NoRC: 20 nM, RSF: 10 nM, NURF: 50 nM, BRG1: 370 nM, CHD4: 200 nM.

DNA preparation

Barcoded 601 (BC-601) DNA preparation

A PstI restriction site was introduced into a 147 b.p. 601 sequence via site-directed mutagenesis to detect nucleosome remodeling. The 601 sequence contains 5'-DrallI and 3'-BsaI overhangs and was prepared by digestion of a plasmid containing 16 copies of the desired sequence; full sequence of one repetitive unit:

5'-

**GTGACAGGATGTATATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTT
GGCGGTTAAACGCGGGGGACAGCGCGTACGTGCGTTTAAGCGGTGCTAGAGC
TGTCTACGACCAATTGAGCGGCTGCAGCACCGGGATTCTCCAG – 3'**

601 sequence – bold

PstI site – underlined (CTGCAG)

The plasmid was produced in and purified from DH5 α cells as previously described³⁸. Approximately 20 mg of plasmid was obtained from 6 L of bacterial culture. 10 mg of the plasmid was then digested with BsaI and DrallI (total DNA concentration of 1 mg/mL in the presence of 500 U/mL of each restriction enzyme) at 37 °C overnight. Digested DNA was purified by phenol chloroform extraction and concentrated by ethanol precipitation. The pellet was resuspended in roughly 1 mL of TE buffer (50 mM Tris, 0.1 mM EDTA, pH 7.5) and sucrose was added to a final concentration of 15%. The desired fragment was then purified by polyacrylamide gel electrophoresis (5% polyacrylamide gel, 0.5x TBE buffer) using a preparative cell (Bio-Rad). A peristaltic pump in-line with a Foxy R1 fraction collector (Teledyne Isco) was used to collect eluting fractions every 45 seconds at a flow rate of 1 mL/minute. Fractions containing the desired fragment were pooled and concentrated by ethanol precipitation. The pellet was resuspended in water yielding approximately 1-2 mg of 601 DNA. Unique nucleosome identifier barcodes were then individually ligated to 601 DNA fragment using the non-palindromic 5'-DrallI site. Individual oligo pairs were purchased from Integrated DNA Technologies. Each oligo was dissolved in TE buffer to a concentration of roughly 100 μ M. For hybridization, equimolar amounts of oligo pairs (top and bottom strand) were mixed at a concentration of 10 μ M each oligo in a volume of 500 μ L. Samples were heated on a heat block for 5 minutes at 95 °C, and were subsequently left to cool slowly by placing the heat block on a lab benchtop at room temperature for 1 hour. Individual oligos contained the following sequence:

Top strand – 5' – **CTCTTTCCCTACACGACGCTCTTCCGATCT**NNNNNNCACCGC – 3'
 Bottom strand – 3' – **GTGNNNNNN**AGATCGGAAGAGCGTCGTGTAGGGAAAGAGAA** – 5'**

Partial Illumina forward adapter sequence – bold

Unique hexanucleotide nucleosome identifier barcode – NNNNNN

A list of the unique hexanucleotide nucleosome identifier barcodes used in this study can be found in Supplementary Table 5. Relative to the top strand, hybridization resulted in double-stranded BC DNA with a 3' overhang compatible with ligation to the 5'-Drall overhang on the purified 601 sequence and a 3'-AA dinucleotide overhang to prevent blunt-end ligation of hybridized oligos. In a typical ligation reaction 0.41 μ M of 601 DNA was combined with 1.2 equivalents of double stranded BC DNA and incubated at 37 °C with 0.1 U/ μ L T4 polynucleotide kinase (NEB) in a total volume of 100 μ L in 1x T4 ligase buffer (NEB) for 1 hour. Subsequently 5 U/ μ L T4 DNA ligase were added, and the mixture was incubated for 1 hour at room temperature. Ligation reactions were monitored by native polyacrylamide gel electrophoresis (5% polyacrylamide gel, 0.5x TBE, 200 V, 40 minutes) and staining with SYBR® Safe DNA gel stain (ThermoFisher Scientific; Extended Data Fig. 2a). The final product was purified using a Qiagen PCR purification kit and quantified by UV spectroscopy at 260 nm. The final product resulted in a 192 b.p DNA fragment composed of the 147 b.p. 601 sequence and a 45 b.p. overhang containing a unique hexanucleotide barcode and a partial Illumina forward adapter sequence:

5' –
CTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNCACCGCGTG**ACAGGATGTA**
TATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTGCGGGTTAAAACG
CGGGGGACAGCGCGTACGTGCGTTTAAGCGGTGCTAGAGCTGTCTACGACCAAT
TGAGCGG**CTGCAG****CACCGGGATTCTCCAG** – 3'

601 sequence – bold

PstI site – underlined (CTGCAG)

Unique hexanucleotide nucleosome identifier barcode – NNNNNN

The partial Illumina forward adapter sequence is the 30 b.p. 5' of the hexanucleotide barcode.

CpG methylated nucleosomal DNA was prepared as described in Extended Data Fig. 2b.

Biotinylated MMTV 'buffer' DNA

Biotinylated MMTV 'buffer' DNA was prepared by PCR using a MMTV DNA template³⁹ with the following primers purchased from Integrated DNA technologies:

Forward – 5' – biotin-TATCACTTGCAACAGTCCTAACATTACCTC – 3' (HPLC purified)

Reverse – 5' – ATCCAAAAAAGTGTGCCGCAGTCGG – 3'

The PCR product was purified using a Qiagen PCR purification kit. The final product was quantified by UV spectroscopy at 260 nm and stored at -20 °C.

192 b.p. DNA fragment used in follow-up studies on individual nucleosomes

601 DNA used in non-library remodeling assays on individual nucleosomes was generated via PCR. The 147 b.p. 601 sequence containing a PstI site was inserted into a KS BlueWhite screening vector using standard restriction enzyme techniques. Primers were designed to amplify the 601 sequence with a 45 b.p. overhang generated from the surrounding vector sequence.

Forward – 5' - GGC CGC TCT AGA ACT AGT – 3'

Reverse – 5' - CTG GAG AAT CCC GGT – 3'

DNA fragment generated:

GGCCGCTCTAGAACTAGTGGATCCGATATCGCTGTTACCGCGTG**ACAGGATGTA**
TATATCTGACACGTGCCTGGAGACTAGGGAGTAATCCCCTTGCGGGTTAAACG
CGGGGGACAGCGCGTACGTGCGTTTAAGCGGTGCTAGAGCTGTCTACGACCAAT
TGAGCGGCTGCTGCAG**CACCGGGATTCTCCAG**

601 sequence – bold

PstI site – underlined (CTGCAG)

Octamer formation for the nucleosome library

Octamers containing desired histone compositions were assembled as previously described¹⁸ with minor modifications. In brief, histones were dissolved in histone unfolding buffer (6 M guanidine hydrochloride, 20 mM Tris, 5 mM DTT, pH 7.5 at 4 °C) and combined in equimolar ratios (0.75 nmol each of desired version of histones H2A, H2B, H3, and H4). The total histone concentration was adjusted to 1 mg/mL, and the mixtures were placed in Slide-A-Lyzer MINI dialysis devices (3.5 kDa MW cutoff, ThermoFisher Scientific) and dialyzed at 4 °C against 3 x 400 mL of octamer refolding buffer (2 M NaCl, 10 mM Tris, 0.5 mM EDTA, 1 mM DTT, pH 7.8 at 4 °C) for at least 4 hours for each step, with one dialysis step overnight. The mixtures were then transferred to clean microcentrifuge tubes and spun down at 17,000 x g for 5 minutes at 4 °C to remove any precipitate. Supernatants were transferred to fresh microcentrifuge tubes, and 50% (v/v) glycerol was added. Octamer concentrations were measured by UV spectroscopy at 280 nm, and stored at -20 °C until use in nucleosome assembly.

Nucleosome reconstitution for the nucleosome library

Nucleosomes were assembled as previously described¹⁸ with minor modifications. In brief, in a typical nucleosome assembly, a crude histone octamer preparation (50 pmol) was combined with the appropriate BC-601 DNA (25 pmol) and biotinylated MMTV 'buffer' DNA (25 pmol) in 70 μ L of octamer refolding buffer (2 M NaCl, 10 mM Tris, 0.5 mM EDTA, 1 mM DTT, pH 7.8 at 4 °C). Mixtures were placed in Slide-A-Lyzer MINI dialysis devices (3.5 kDa MW cutoff, ThermoFisher Scientific) and dialyzed at 4 °C against 200 mL nucleosome assembly start buffer (10 mM Tris, 1.4 M KCl, 0.1 mM EDTA, 1 mM DTT, pH 7.8 at 4 °C) for 1 hour at 4 °C. Subsequently, 330 mL of nucleosome assembly end buffer (10 mM Tris, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, pH 7.8 at 4 °C) were added at a rate of 1 mL/minute using a peristaltic pump, followed by two final dialysis steps against nucleosome assembly end buffer (1 hour and overnight). The dialysis mixture was transferred to a microcentrifuge tube, and removal of biotinylated MMTV 'buffer' DNA-associated side products was accomplished by affinity depletion using 140 μ L of MyOne streptavidin T1 coated Dynabead slurry (ThermoFisher Scientific) for 1 hour at room temperature. The unbound material in the supernatant was transferred to a clean microcentrifuge tube, and any precipitate was removed by centrifugation. Final nucleosome preparations were quantified by UV spectroscopy at 260 nm. The quality of individual nucleosomes was assessed by native polyacrylamide gel electrophoresis (5% acrylamide gel, 0.5x TBE, 200 V, 40 minutes), followed by ethidium bromide staining. This resulted in a main band migrating around 500 b.p. (variable depending on the nucleosome variant; Extended Data Fig. 3a). To form the library, nucleosome preparations were directly combined and concentrated using Vivaspin 500 centrifugal filter units (10 kDa MW cutoff, Vivaproducs). The final nucleosome library was analyzed by native gel electrophoresis as previously described (Extended Data Fig. 3b). For storage, 20% (v/v) glycerol was added resulting in a final library concentration of approximately 1.5 μ M. Library aliquots were flash frozen in liquid nitrogen, and stored at -80 °C until being thawed before use.

Analysis of nucleosome library integrity by antibody pull-down

We assessed nucleosome library integrity by antibody pull-down against a specific histone mark. The nucleosome library (12 fmol of each member) was combined with a modification specific antibody (anti-H3K4me3, Abcam: ab8580; 15 μ g/mL final concentration) in 100 μ L of antibody binding buffer (20 mM Tris, 50 mM NaCl, 5 mM EDTA, 0.1% TWEEN® 20) and incubated at room temperature for 1 hour. The volume was then brought to a final volume of 200 μ L with antibody binding buffer. 10 μ L of Pierce Protein G agarose slurry (previously washed with antibody binding buffer) were then added and the mixture was incubated for an additional hour at room temperature to allow antibody binding to the Protein G beads. The beads were then washed 4 times with antibody binding buffer and incubated with 100 μ L of elution buffer (100 mM Tris, pH 7.5, 10 mM EDTA, 1% SDS, 10 mM, 2-mercaptoethanol, 8 U/mL Proteinase K) for 90 minutes at 50 °C. DNA was purified using a Qiagen PCR purification kit, and was subsequently quantified using a Qubit high-sensitivity dsDNA quantification kit. DNA was diluted with water to a final concentration of approximately 2 pg/ μ L. The sample was PCR amplified according to conditions described in the methods section 'Addition

of multiplex barcodes and Illumina forward and reverse adapter sequences via PCR'. An input sample was prepared by directly mixing an equivalent amount of nucleosome library with elution buffer followed by identical sample processing. Sequencing was performed as described in the section 'Illumina sequencing'. Experimental samples were normalized against the input, and, within the experiment, the indicated variant (Extended Data Fig. 3c).

Nucleosome thermal mobility shift experiments

Nucleosome thermal mobility shift experiments were performed similarly to that previously described³⁹. Octamers and nucleosomes were prepared similarly to that previously described³⁸ with minor modifications. Nucleosomes were assembled via salt gradient dialysis on a DNA fragment that was PCR amplified from a pTF vector to generate a 283 b.p. fragment containing a centrally positioned MMTV sequence (NucA)³⁹ with 64 b.p. of flanking DNA on each side.

Forward: 5' - ATT TAT TAT GCA TTT AGA ATA AAT TTT GTG TCG CCC TTG – 3'

Reverse: 5' - CAG TCG AAA GAC TGG GCC TTT C – 3'

DNA fragment generated:

5' —
 ATTTATTATGCATTTAGAAATAAATTTTGTGTCGCCCTTGTGCTGAGGTACCAGATC
 TGATATCACTTGCAACAGTCCTAACATTACCTCTTGTGTGTTTGTGTCTGTTTCGC
CATCCCGTCTCCGCTCGTCACTTATCCTTCACTTTCCAGAGGGTCCCCCGCAGA
CCCCGGCGACCTGGTCGGCCGACTGCGGGCACAGTTTTTTGGATATCGGATCCC
 GTCAATCGAGAAGGGCGACACCCCTAATTAGCCCGGGCGAAAGGCCCAGTCTTT
 CGACTG – 3'

MMTV sequence – bold

Approximately 1 pmol of each nucleosome type were incubated in assay buffer (50 mM Tris, 150 mM NaCl, pH 7.5) for 1 hour at either 37 °C or 47 °C. After incubation, 5 µL of 50% sucrose were added to each sample and they were run on a 5% TBE gel in 0.5x TBE buffer for 40 minutes at 200V. Nucleosomes were visualized by staining with SYBR® Gold Nucleic Acid Gel Stain (ThermoFisher Scientific). Unmodified and H3K14cr nucleosomes are not expected to be as mobile as H3T118H and H4R45A nucleosomes, which contain mutations that affect interactions between histones and DNA.

Experiments employing the nucleosome library

Remodeling assays using the nucleosome library

Remodeling assays employed a restriction enzyme accessibility (REA)-based strategy and were performed similarly to those previously described¹⁹. In each

remodeling reaction, 10 nM of library was used along with varying amounts of remodeling enzyme depending on the baseline activity of each protein preparation. Typically, an amount of remodeler that resulted in approximately 50% of unmodified nucleosomes remodeled in 1 hour was used (200 nM SNF2h, 2 nM ACF, 5 nM CHRAC, 10 nM WICH, 5 nM NoRC, 40 nM RSF, and 50 nM NURF). 50 μ L remodeling assays were carried out in REA buffer (12 mM HEPES, pH 7.9, 4 mM Tris, pH 7.5, 60 mM KCl, 10 mM MgCl₂, 10% glycerol, and 0.02% (v/v) IGEPAL CA-630) with or without 2 mM ATP and in the presence of 2 U/ μ L PstI restriction enzyme (NEB). Reactions were pre-incubated for 10 minutes at 30 °C prior to initiation by the addition of nucleosomes. Note that ATP (or an equivalent volume of REA buffer in the case of reactions without ATP) was not added until approximately 2.5 minutes prior to initiation. After initiation, remodeling assays were carried out for 1 hour at 30 °C. 6 μ L time points were taken at 1, 2.5, 5, 15, 30, and 60 minutes and each was quenched with 9 μ L of quench buffer (10% glycerol, 70 mM EDTA, pH 8, 20 mM Tris, pH 7.7, 2% SDS, 0.2 mg/mL bromophenol blue). Samples were deproteinized with 30 U/mL Proteinase K (NEB) for 1 hour at 37 °C. DNA was purified using a Qiagen PCR purification kit, eluting in 50 μ L of TE buffer (50 mM Tris, 0.1 mM EDTA, pH 7.5). For each enzyme, reactions with and without ATP were performed in triplicate. With 6 time points taken per reaction, this generated 36 samples per enzyme analyzed. Samples were quantified using a Qubit high-sensitivity dsDNA quantification kit. DNA was diluted with water to a final concentration of approximately 2 pg/ μ L. The sample was then PCR amplified as per conditions described in the methods section 'Addition of multiplex barcodes and Illumina forward and reverse adapter sequences via PCR'.

Pull-down experiments with the RCC1-GST construct using the nucleosome library

10 μ L of Pierce Glutathione Agarose slurry were washed 4 times with 100 μ L of binding buffer (20 mM Tris, 50 mM NaCl, 5 mM EDTA 0.1% TWEEN® 20, pH 7.5). The beads were resuspended in 100 μ L of binding buffer after the final wash and 1.5 pmol of RCC1-GST were added. Binding to the beads was allowed to occur for 1.5 hours at room temperature with end-over-end rotation. After incubation, the beads were washed again 4 times with 100 μ L of binding buffer. The beads were resuspended in 100 μ L of binding buffer after the final wash and combined with the nucleosome library (12 fmol of each member). After incubation with end-over-end rotation for 2 hours at 4 °C, the beads were washed again 4 times with 100 μ L of binding buffer. The beads were then directly resuspended in 100 μ L of DNA elution buffer (100 mM Tris, 10 mM EDTA, 1% SDS, 10 mM β -mercaptoethanol, 200 μ g/mL Proteinase K) and incubated for 1.5 hours at 50 °C. At this point an input sample for normalization was prepared by taking a small amount of nucleosome library, resuspending it in 100 μ L of DNA elution buffer, and incubating for 1.5 hours at 50 °C. DNA from all samples was purified using a Qiagen PCR purification kit, eluting in 50 μ L of TE buffer (50 mM Tris, 0.1 mM EDTA, pH 7.5). Samples were quantified using a Qubit high-sensitivity dsDNA quantification kit. DNA was diluted with water to a final concentration of approximately 2 pg/ μ L. The sample was then PCR amplified as per conditions described in the methods section 'Addition of multiplex barcodes and Illumina forward and reverse adapter sequences via PCR'.

Pull-down experiments with the Sir3-BAH-FLAG-6xHis construct using the nucleosome library

10 μ L of ANTI-FLAG® M2 (Sigma-Aldrich) slurry were washed 4 times with 100 μ L of binding buffer (20 mM Tris, 50 mM NaCl, 5 mM EDTA 0.1% TWEEN® 20, pH 7.5). The beads were resuspended in 50 μ L of binding buffer after the final wash and 40 pmol of Sir3-BAH-FLAG-6xHis were added. Binding to the beads was allowed to occur for 1 hour at room temperature with end-over-end rotation. After incubation, the beads were washed again 4 times with 50 μ L of binding buffer. The beads were resuspended in 40 μ L of binding buffer after the final wash and combined with the nucleosome library at a final concentration of 40 nM. After incubation with end-over-end rotation for 1 hour at room temperature, the beads were washed again 4 times with 50 μ L of binding buffer. The beads were then directly resuspended in 100 μ L of DNA elution buffer (100 mM Tris, 10 mM EDTA, 1% SDS, 10 mM β -mercaptoethanol, 200 μ g/mL Proteinase K) and incubated for 1.5 hours at 50 °C. At this point an input sample for normalization was prepared by taking a small amount of nucleosome library, resuspending it in 100 μ L of DNA elution buffer, and incubating for 1.5 hours at 50 °C. DNA from all samples was purified using a Qiagen PCR purification kit, eluting in 50 μ L of TE buffer (50 mM Tris, 0.1 mM EDTA, pH 7.5). Samples were quantified using a Qubit high-sensitivity dsDNA quantification kit. DNA was diluted with water to a final concentration of approximately 2 pg/ μ L. The sample was then PCR amplified as per conditions described in the methods section 'Addition of multiplex barcodes and Illumina forward and reverse adapter sequences via PCR'.

Addition of multiplex barcodes and Illumina forward and reverse adapter sequences via PCR

Approximately 10 pg of DNA from each purified experimental sample collected from remodeling assays or binding experiments using the nucleosome library were added to a PCR reaction to be amplified using the following primer pair:

Forward primer

5' – AATGATACGGCGACCAACGAGATCTACACTCTTTCCCTACACGACG – 3'

Reverse primer

5' – CAAGCAGAAGACGGCATACGAGATXXXXXXCTGGAGAATCCCGGTG – 3'

Unique hexanucleotide multiplexing barcode – XXXXXX

A list of the unique hexanucleotide multiplexing barcodes used in this study can be found in Supplementary Table 6. Specific amplification of full-length (un-cut) nucleosomal DNA at each assay time point was carried out using a Phusion® High-

Fidelity PCR kit from NEB and primers flanking the PstI restriction cut site (1 mM dNTPs, 10 pg DNA, 0.5 μ M each primer, 0.02 U/ μ L polymerase) with the following amplification conditions: Step 1: 98 °C for 30 seconds, Step 2: 98 °C for 10 seconds, Step 3: 47 °C for 15 seconds, and Step 4: 72 °C for 8 seconds. Steps 2-4 were repeated for a total of 15 cycles followed by a final extension step at 72 °C for 7 minutes. To ensure reactions remained in the exponential phase and relative abundances of DNA fragments were maintained, qPCR analysis was performed using 10 pg of a chosen remodeling reaction time point using SYBR Green I Dye for detection on an ABI 7900 quantitative PCR instrument. Identical conditions were used as previously described except a 10 second extension time was used (instead of 8 seconds) due to the instrument requirements. A mean C_t value of 13.9 was determined (compare to 15 cycles used for amplification of DNA prior to Illumina sequencing). The following amplicon was generated that is compatible with Illumina sequencing:

5' —
AATGATACGGCGACCAACGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGAT
CTNNNNNNCACCGCGTG**ACAGGATGTATATATCTGACACGTGCCTGGAGACTAG**
GGAGTAATCCCCTTGGCGGTTAAACGCGGGGGACAGCGCGTACGTGCGTTTAA
GCGGTGCTAGAGCTGTCTACGACCAATTGAGCGGCTGCAGCACCGGGATTCTCC
AGXXXXXXATCTCGTATGCCGTCTTCTGCTTG – 3'

601 sequence – bold

PstI site – underlined (CTGCAG)

Unique hexanucleotide nucleosome identifier barcode – NNNNNN

Unique hexanucleotide multiplexing barcode – XXXXXX

The full Illumina forward adapter sequence is 5' of the unique hexanucleotide nucleosome identifier barcode (NNNNNN). The full Illumina reverse adapter sequence is 3' of the unique hexanucleotide multiplexing barcode (XXXXXX). Samples containing unique multiplexing barcodes could be pooled directly after PCR and purified using a Qiagen PCR purification kit in one pot. After purification, samples were subjected to high-throughput DNA sequencing as described in the methods section 'Illumina sequencing'. In each case, the correct amplicon size (250 b.p.) was verified by agarose gel electrophoresis and staining with EtBr.

Illumina sequencing

Single-end sequencing of barcoded DNA libraries (starting from the forward adapter and covering the unique nucleosome identifier barcode) was performed by the Lewis Sigler Institute for Integrative Genomics Sequencing Core Facility at Princeton University on an Illumina HiSeq 2500 with read length of 67 b.p. Due to the significant sequence homogeneity present in samples generated from remodeling experiments, these libraries were diluted with a PhiX control library to enable sequencing. A custom sequencing primer was used to sequence the index read (5' – CAATTGAGCGGCTGCAGCACCGGGATTCTCCAG – 3'). This primer annealed to the 601 sequence and the 8 b.p. index read covered the unique multiplexing barcode.

Nucleosome remodeling experiments in the presence of LANA peptides

Remodeling experiments in the presence of LANA and LANA mutant (LRS to AAA) peptides and rate calculations were performed as described in the methods section 'Nucleosome remodeling rate analysis for validation experiments on single nucleosomes'. LANA peptides were pre-incubated with unmodified nucleosomes for 10 minutes on ice before being used in remodeling assays at a final concentration of 10 μ M peptide.

Nucleosome remodeling rate analysis for validation experiments on individual nucleosomes using a restriction enzyme accessibility assay

All remodeling experiments validating results obtained from the nucleosome library were performed on nucleosomes assembled with a 192 b.p. fragment described in the methods section '192 b.p. DNA fragment used in follow-up studies on single nucleosomes'. Remodeling assays were performed as described in the methods section 'Remodeling assays using the nucleosome library' with some modifications. Experiments involving the NURF complex were carried out using nucleosomes at 10 nM and NURF at 50 nM. Experiments involving the ACF complex were performed using nucleosomes at 10 nM and ACF at 2 nM. Experiments involving CHD4 were carried out using nucleosomes at 10 nM and CHD4 at 5 nM. Experiments involving BRG1 were carried out using nucleosomes at 10 nM and BRG1 at 200 nM. All reactions were carried out in the presence of 2 mM ATP. Time points were taken, quenched, and deproteinized as described in the methods section 'Remodeling assays using the nucleosome library'. Samples were directly run on a 5% polyacrylamide gel (0.5x TBE, 200 V, 40 minutes). Staining was performed with SYBR® Safe DNA gel stain and gels were imaged on a Typhoon scanner (GE Healthcare). Densitometry measurements were performed using Image Studio Lite (LI-COR). Rates were determined using GraphPad Prism by fitting to a single exponential decay equation (see Extended Data Fig. 9a, c, d, and Supplementary Fig. 5, and methods section 'Nucleosome remodeling rate analysis for library experiments').

Nucleosome remodeling assays and rate analysis for validation of acidic patch mutant nucleosomes (H2A E61A, D90A, E92) using an electrophoretic mobility shift nucleosome repositioning assay

Octamers and nucleosomes were prepared similarly to previously described³⁸ with minor modifications. Nucleosomes were assembled via salt gradient dialysis on a 227 b.p. DNA fragment that was prepared similarly as in the methods section '192 b.p. DNA fragment used in follow-up studies on individual nucleosomes'. In this case, primers were designed to amplify the 601 sequence with a 80 b.p. overhang generated from the surrounding vector sequence.

Forward – 5' - CAC TAT AGG GCG AAT TGG AG – 3'

Reverse – 5' - CTG GAG AAT CCC GGT – 3'

DNA fragment generated:

CACTATAGGGCGAATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTG
GATCCGATATCGCTGTTACCGCGTG**ACAGGATGTATATATCTGACACGTGCCTG**
GAGACTAGGGAGTAATCCCCTTGGCGGTTAAACGCGGGGGACAGCGCGTACG
TGCGTTTAAGCGGTGCTAGAGCTGTCTACGACCAATTGAGCGGCTGCAGCACCG
GGATTCTCCAG

601 sequence – bold

50 μ L remodeling assays were carried out in assay buffer (12 mM HEPES, pH 7.9, 4 mM Tris, pH 7.5, 60 mM KCl, 10 mM $MgCl_2$, 10% glycerol, and 0.02% (v/v) IGEPAL CA-630) with 30 nM ACF complex and 90 nM nucleosomes. Reactions were pre-incubated for 10 minutes at 30 °C prior to initiation by the addition of nucleosomes. Note that ATP (final concentration equivalent to 2 mM) was not added until approximately 2.5 minutes prior to initiation. After initiation, remodeling assays were carried out for 1 hour at 30 °C. 6 μ L time points were taken at 1, 2.5, 5, 15, 30, and 60 minutes and each was quenched by addition of 6 μ L of quench buffer (assay buffer with 800 ng/ μ L sheared salmon sperm DNA (ThermoFisher Scientific)) and placement on ice. Quenched assay samples were directly run on a 5% TBE gel in 0.5x TBE buffer for 40 minutes at 200V. Nucleosomes were visualized by staining with SYBR® Gold Nucleic Acid Gel Stain (ThermoFisher Scientific).

Densitometry measurements were performed to quantify the movement of nucleosomes away from their initial position using Image Studio Lite (LI-COR). Rates were determined using GraphPad Prism by fitting to a single exponential decay equation (see Extended Data Fig. 9b).

Processing of sequencing data for chromatin remodeling experiments using the nucleosome library

As previously noted, each remodeling experiment (per enzyme) generated 36 samples (triplicate reactions with and without ATP with 6 time points collected per reaction). Individual remodeling experiments were processed as follows: 67 b.p. single-end and 8 b.p. index reads were imported into Galaxy (Princeton University installation). Corresponding read pairs (single-end and index) generated from the same fragment were joined to generate single nucleotide sequences that contained both a unique nucleosome identifier barcode and related unique multiplexing barcode. All reads were then split into separate FASTQ files (36 in total) based on their unique multiplexing barcodes using Galaxy's Barcode Splitter tool. Each FASTQ file was exported from Galaxy, and occurrences of each unique nucleosome identifier barcode within each file were counted using a custom R script. This resulted in individual data points composed of a distinct number of read counts associated with a unique nucleosome identifier barcode and a unique multiplexing barcode.

Also, included in the nucleosome library was a non-nucleosomal DNA fragment identical to BC-601 DNA except that it did not contain a PstI site (DNA Standard 1). It

was carried with the sample from the initiation of remodeling experiments through Illumina sequencing, and used as an internal reference to normalize relative abundances of DNA between multiplexed samples. After normalization, sample read counts were organized by time point/experimental condition (with or without ATP) and nucleosome type (Supplementary Table 2). At this point, nucleosomes 71, 78, and 113 were removed from further analysis as their unique nucleosome identifier barcodes created an additional PstI site with the surrounding nucleotide sequence.

Processing of sequencing data for binding experiments using the nucleosome library

Barcode sorting was performed as described in the methods section ‘Processing of sequencing data for chromatin remodeling experiments using the nucleosome library’. To account for variation in abundance of individual library members, raw read counts (Supplementary Table 7) for each nucleosome were first normalized to corresponding values from the input sample. Note that data from a single input sample was used for processing all pull-down experiments. To better visualize how nucleosome modifications or mutations affect binding, these values were then normalized to one of the unmodified nucleosomes in the nucleosome library (nucleosome 42, see Supplementary Table 1) to calculate relative affinity measurements for all nucleosome library members.

Nucleosome remodeling rate analysis for library experiments

For each enzyme, remodeling rate constants per nucleosome were calculated for experimental triplicates in GraphPad Prism. Also, included in the nucleosome library was a non-nucleosomal DNA fragment identical to BC-601 DNA (DNA Standard 2). Nucleosome remodeling data were fit to a 2-phase exponential decay equation (see below). The first phase (k_{fast}) was fixed as the rate of cutting of DNA Standard 2 in each experiment (fit to 1-phase exponential decay equation). The y-intercept was allowed to vary and the plateau was set to zero. Fitting to this model allowed the second phase (k_{slow}) to be determined as the rate of nucleosome remodeling (this parameter was left unconstrained) (see Supplementary Fig. 5 and below). Additionally, to estimate the minimum rate of remodeling capable of being determined by our analysis methods we averaged the bottom 1% of rates that had 95% confidence interval non-overlapping with zero and calculated the associated error. Any values below this threshold were set equal to it. This included cases where k_{slow} was extremely slow (the curve very flat) and determined to be slightly negative. The remodeling rates of unmodified nucleosomes in the library were then averaged and the associated error was calculated. \log_2 (fold-change) values relative to the unmodified nucleosome rate average and associated errors were then computed for each nucleosome in the library. Singular value decomposition of the nucleosome library data was calculated for PCA with the Python module matplotlib.mlab (v. 1.4.3).

Fitting the remodeling kinetic data to a 2-phase decay was necessary to account for the presence of free BC-601 DNA for some members in the library – in particular nucleosomes containing polyacetylated H3 and H4 (Supplementary Fig. 5b). Use of a 1-

phase exponential decay led to an overestimation of the rate constants (and a poorer fit) for several library members, due to the contribution of the free DNA which is rapidly cut by the PstI restriction enzyme, irrespective of remodeling activity (Supplementary Fig. 5a, c). Fitting the data to the 2-phase exponential led to a better fit for those library members. The first phase accounts for the free DNA, whereas the slower second phase reports on the remodeling rate of the nucleosome. Note, the vast majority of library members had minimal free BC-601 DNA and could be analyzed equally well using 1- or 2-phase decays (Supplementary Fig. 5e, f). For consistency, we used the 2-phase decay fit throughout. Contributions of free DNA to rate measurements were generally not thought to be dependent on systematic irreproducibility in the nucleosome assembly procedures used in this study, but typically on the relative affinities of certain modified histone octamers for nucleosomal DNA as observed by gel electrophoresis. For example, polyacetylated nucleosomes tended to show such behavior (Supplementary Fig. 5b). We therefore propose that the amount of free DNA in a nucleosome preparation must be carefully considered when fitting nucleosome remodeling data generated by a restriction enzyme accessibility assay to a 1-phase exponential decay equation. In the case, where an internal standard to measure the rate of cutting of non-nucleosomal DNA is present, (DNA standard 2 in our high-throughput nucleosome remodeling assay) data may be better modeled by a 2-phase exponential decay equation to aid in accounting for the presence of any free DNA. This proves advantageous in situations where large numbers of nucleosomes must be prepared and precious material is limited or if nucleosome assembly reactions prove difficult to optimize.

Code availability

A custom R script was used to count individual DNA barcodes in the processing of sequencing data. Requests to access this code may be sent to Tom W. Muir (muir@princeton.edu).

Data availability

The authors declare that all data supporting the findings of this study are available from the authors and are included within the paper, extended data, and its supplementary information files.

EXTENDED DATA FIGURE LEGENDS

Extended Data Figure 1 | Characterization of Histones by RP-HPLC/ESI-MS.

(Left) Analytical C-18 RP-HPLC chromatograms (insets; gradients used are individually noted) and corresponding ESI-MS spectra of indicated histones prepared in this study. Corresponding deconvoluted spectra are shown on the right. Lysine acetylation: Kac; lysine mono, di, and trimethylation: Kme, Kme2 and Kme3, respectively; arginine mono, asymmetric and symmetric demethylation: Rme, Rme2a and Rme2s, respectively; serine and tyrosine phosphorylation: Sph and Yph, respectively; serine modified with N-acetylglucosamine: SGlcNAc. TFA = trifluoroacetic acid adduct.

Extended Data Figure 2 | Characterization of Barcoded 601 (BC-601) DNA.

a, BC-601 DNA prepared for all 115 nucleosome library members as described in methods section 'Barcoded 601 (BC-601) DNA preparation'. Ligation products are 192 b.p. in size and were visualized by polyacrylamide gel electrophoresis (5% acrylamide, 0.5x TBE, 200 V, 40 minutes) and staining with SYBR® Safe DNA gel stain. A faint band corresponding to unligated 601 DNA (601) is slightly visible in certain cases. **b**, BC-601 DNA for nucleosome 99 (see Supplementary Table 1) was CpG methylated by the M.SssI methyltransferase (NEB) according to the manufacturer's instructions and characterized by digestion with the RsaI restriction enzyme, which is sensitive to CpG methylation, and PstI, which is not. For gel source data, see Supplementary Figure 1.

Extended Data Figure 3 | Analysis of the Quality and Integrity of the Nucleosome Library.

Analysis of individual nucleosome preparations (**a**) and the final library after pooling of nucleosomes (**b**) by native gel electrophoresis and staining with ethidium bromide. **c**, Antibody pull-down of library members using an anti-H3K4me3 antibody. Every nucleosome member containing an H3K4me3 mark (red) was efficiently isolated relative to other library members (black). Notably, the antibody was also able to pull down a nucleosome possessing solely the H3K4me2 mark (blue), indicating a lack of antibody specificity in this case. This experiment was performed once. For gel source data, see Supplementary Figure 1.

Extended Data Figure 4 | Characterization of Recombinant ISWI Chromatin Remodelers.

a, Purified chromatin remodelers were run a 4-20% Mini-PROTEAN® TGX™ gel (Bio-Rad) and for 35 min at 180 V. Proteins were stained with Coomassie. The composition of each remodeler/remodeling complex is depicted above each respective lane on the gel. Expected molecular weights: SNF2h: 122 kDa, ACF1: 179 kDa, CHRAC-15: 14.7 kDa, CHRAC-17: 16.9 kDa, WSTF: 171 kDa, TIP5: 208 kDa, RSF1: 164 kDa; migrates at higher apparent molecular weight, SNF2L: 121 kDa, BPTF: 338 kDa, RbAp46: 47.8 kDa. **b**, All remodelers display ATP-dependent nucleosome remodeling activity as detected by a restriction enzyme accessibility assay. For gel source data, see Supplementary Figure 1.

Extended Data Figure 5 | Nucleosome Remodeling Activity is Negligible in the Absence of ATP.

Bar graphs showing individual DNA cleavage rates (k_{MN} , remodeling rates in the case of nucleosomes; see Supplementary Table 3) from library remodeling experiments for each member of the library in the presence of the indicated chromatin remodeler with and without ATP. Rate values were rank ordered and displayed from low to high. The dashed red line represents the rate of remodeling of unmodified nucleosomes. The related graphs for the ACF complex can be found in Figure 2d. Data are represented as the mean of experimental replicates \pm s.e.m. ($n = 3$).

Extended Data Figure 6 | Principal Component Analysis of Library Remodeling Data.

Principal component (PC) analysis of library remodeling data. Percentages show the fractions of the variance accounted for by each PC. Individual nucleosomes are shown in light blue, and PC weight values for each remodeler are shown in either orange or black. Weights are scaled by a factor of 2 for visibility. **a**, PC1 vs. PC2 and PC1 vs. PC3 are plotted. **b**, PC2 vs. PC3 are plotted as in Fig. 3a. Nucleosomes driving differences in remodeler activity were numbered as in Supplementary Table 1 and grouped by their location in PC space (Supplementary Table 4).

Extended Data Figure 7 | Alteration of Histone-DNA Contacts Affects Remodeling Activity.

a, Modified histone residues in the nucleosome library that lie under the DNA (tan) are highlighted on the nucleosome (PDB: 1KX5) in red. k_{MN} = nucleosome remodeling rate. $k_{unmod.}$ = unmodified nucleosome remodeling rate. PTMs are numbered and labeled on the nucleosome structure. Values were capped at -2 and 2 for display purposes. **b**, Histone mutants present in the nucleosome library that lie under the DNA (tan) are highlighted on the nucleosome (PDB: 1KX5) in red. The heatmap is displayed as in **a**. Locations of each mutation are individually labeled on the nucleosome structure. Values were capped at -3 and 3 for display purposes. All histones are unmodified unless otherwise specified.

Extended Data Figure 8 | Remodeling of Nucleosomes Containing Modifications Preferred by Histone Recognition Domains

Library remodeling data generated by the NoRC (**a**), WICH (**b**), and NURF (**c**) complexes for nucleosomes containing residues known to interact with histone binding modules in accessory subunits of each complex (NoRC: TIP5; WICH: WSTF; NURF: BPTF). Literature binding specificities are displayed in corresponding tables on the right. Bar graphs display \log_2 values of the rate of remodeling of individual nucleosome library members (k_{MN}) relative to unmodified nucleosomes ($k_{unmod.}$). Data are represented as the ratio of the mean of experimental replicates \pm s.e.m. ($n = 3$). Note that H3KpolyAc includes the H3K14ac modification (Supplementary Table 1). All histones are unmodified unless otherwise specified. BRD = bromodomain, PHD = PHD-finger, PHD-BRD = tandem PHD-finger-bromodomain module. ND = not determined.

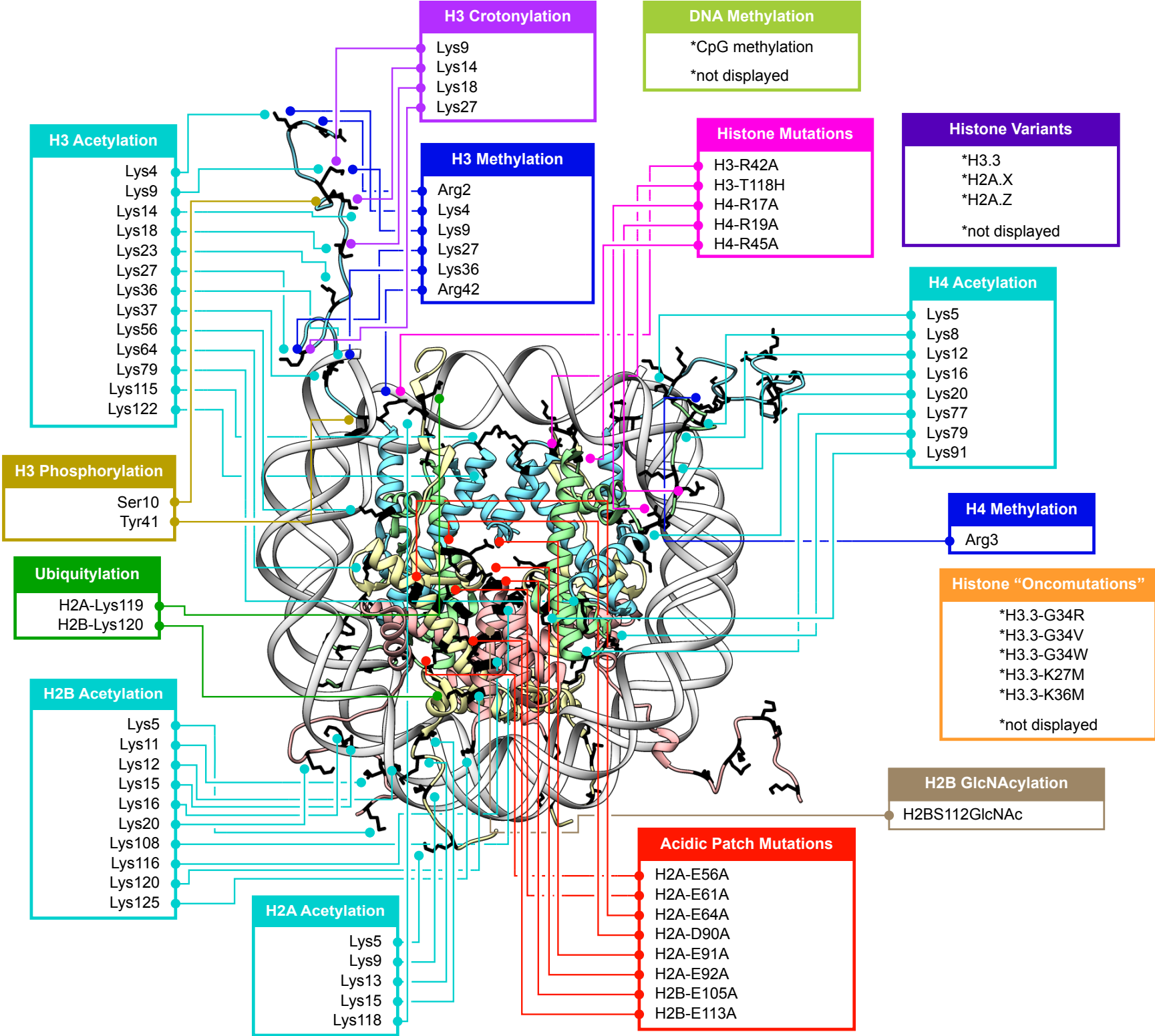
Extended Data Figure 9 | Remodeling Assays Carried out on Individual Nucleosomes Measured via Standard Gel-based Read-out to Validate Library Data.

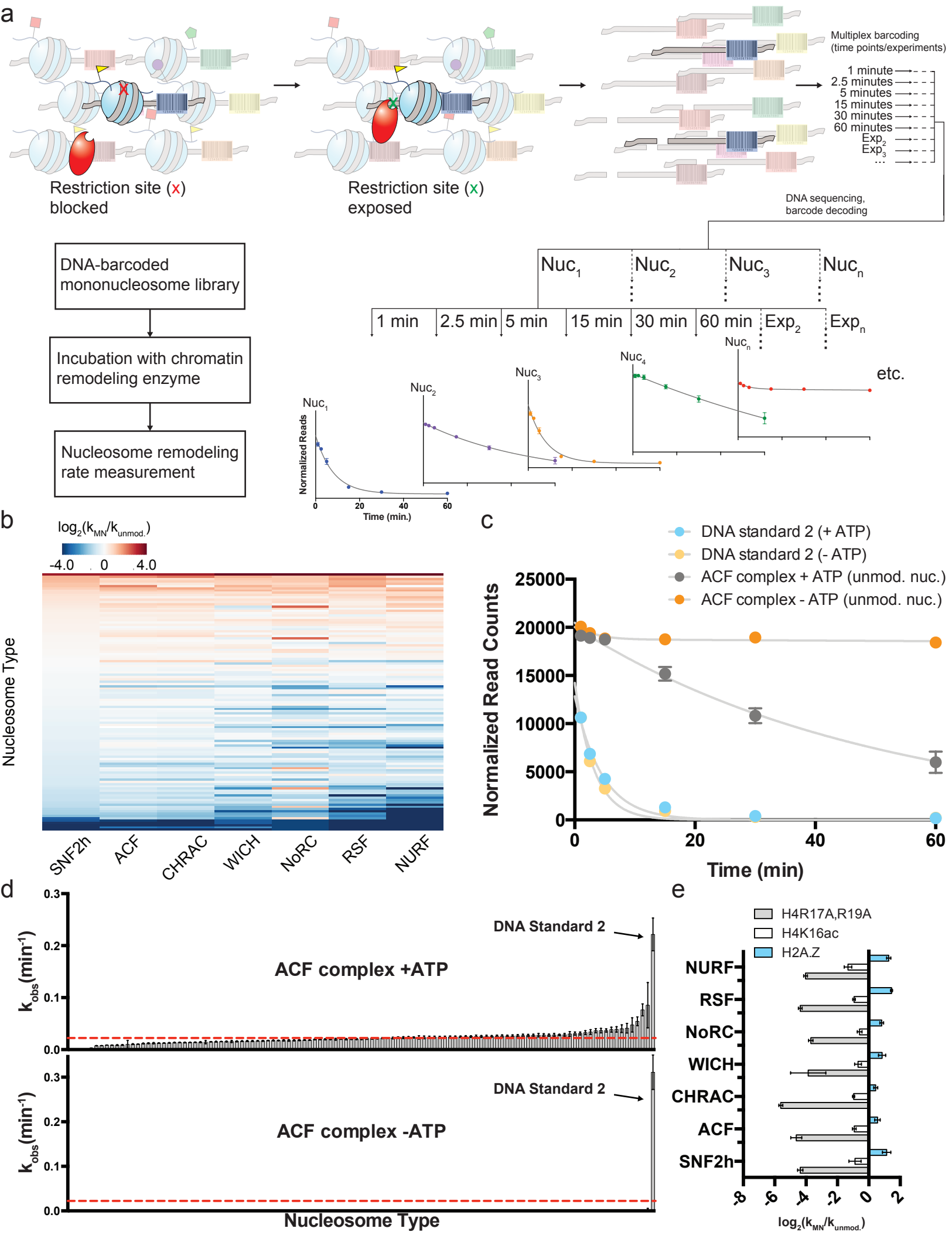
a, Activity of the NURF complex toward H3K4me3+H4K16ac relative to unmodified nucleosomes as measured in the context of the nucleosome library (library) or individual assays (individual). **b**, Activity of the ACF complex on unmodified and acidic patch mutant nucleosomes. **c**, Remodeling of unmodified nucleosomes is inhibited by the presence of the LANA peptide when compared to a LANA peptide with key binding residues mutated (LRS to AAA). **d**, Activity of the ACF complex toward nucleosomes modified near the acidic patch (H2BK108ac and H2BS112GlcNac) relative to unmodified nucleosomes as measured in the context of the nucleosome library (library)

or individual assays (individual). Gel images of example replicates used to generate densitometry measurements in each subpanel are shown above respective graphs. **a**, **c**, and **d** employ a restriction enzyme accessibility assay. **b** employs a nucleosome repositioning electrophoretic mobility shift assay. k_{MN} = nucleosome remodeling rate. $k_{unmod.}$ = unmodified nucleosome remodeling rate. All histones are unmodified unless otherwise specified. All data are represented as the mean of experimental replicates \pm s.e.m. ($n = 3$). For gel source data, see Supplementary Figure 1.

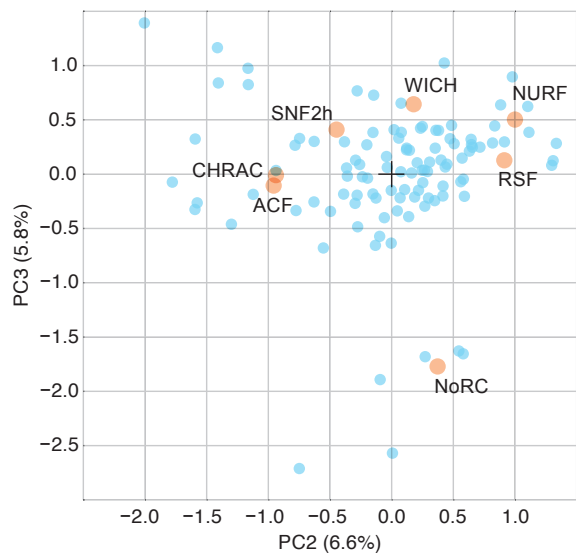
Extended Data Figure 10 | High-throughput Chromatin Remodeling and Binding Data.

a, Heat-map displaying ISWI remodeling data (as in Figure 2b) against the nucleosome library with CHD4 data for comparison. Rows were sorted based on values for SNF2h (low to high). k_{MN} = nucleosome remodeling rate. $k_{unmod.}$ = unmodified nucleosome remodeling rate. **b**, Heat map displaying binding of chromatin factors RCC1 and Sir3 against the nucleosome library relative to unmodified nucleosomes. Values were capped at -4 and 4 for display purposes. All data are represented as the mean of experimental replicates ($n = 3$).

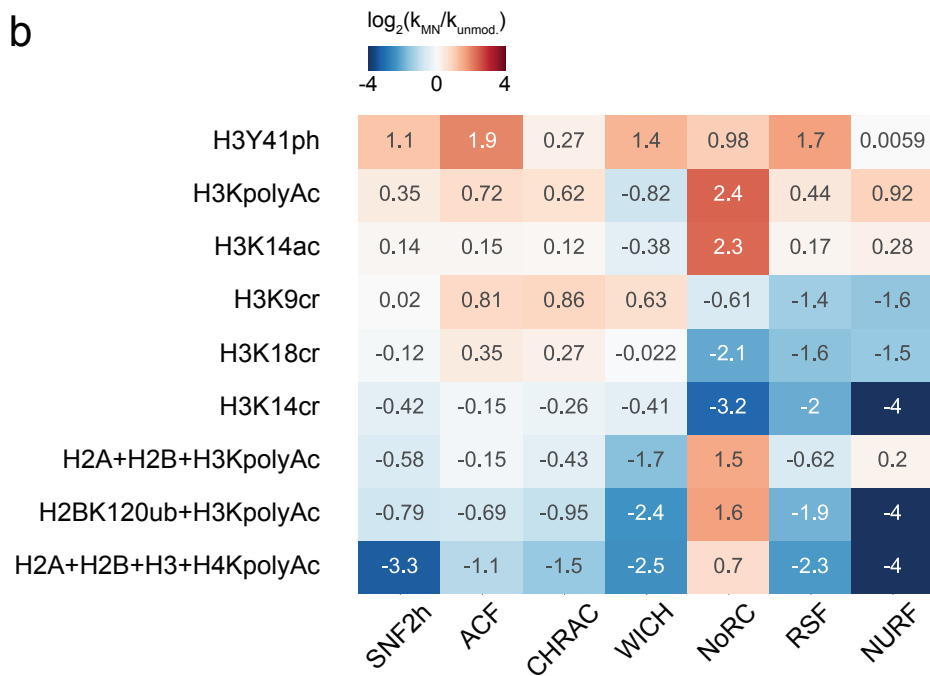




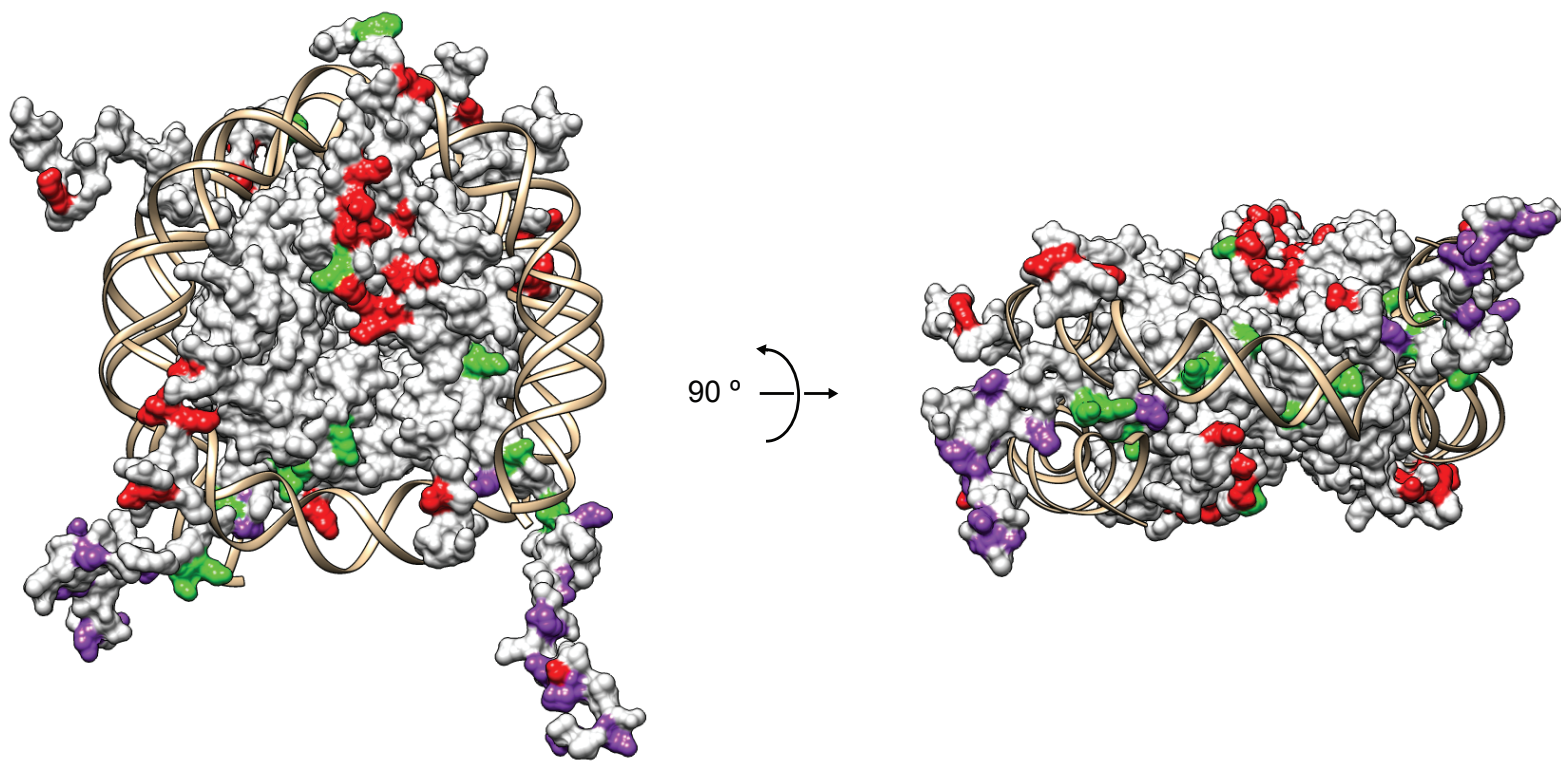
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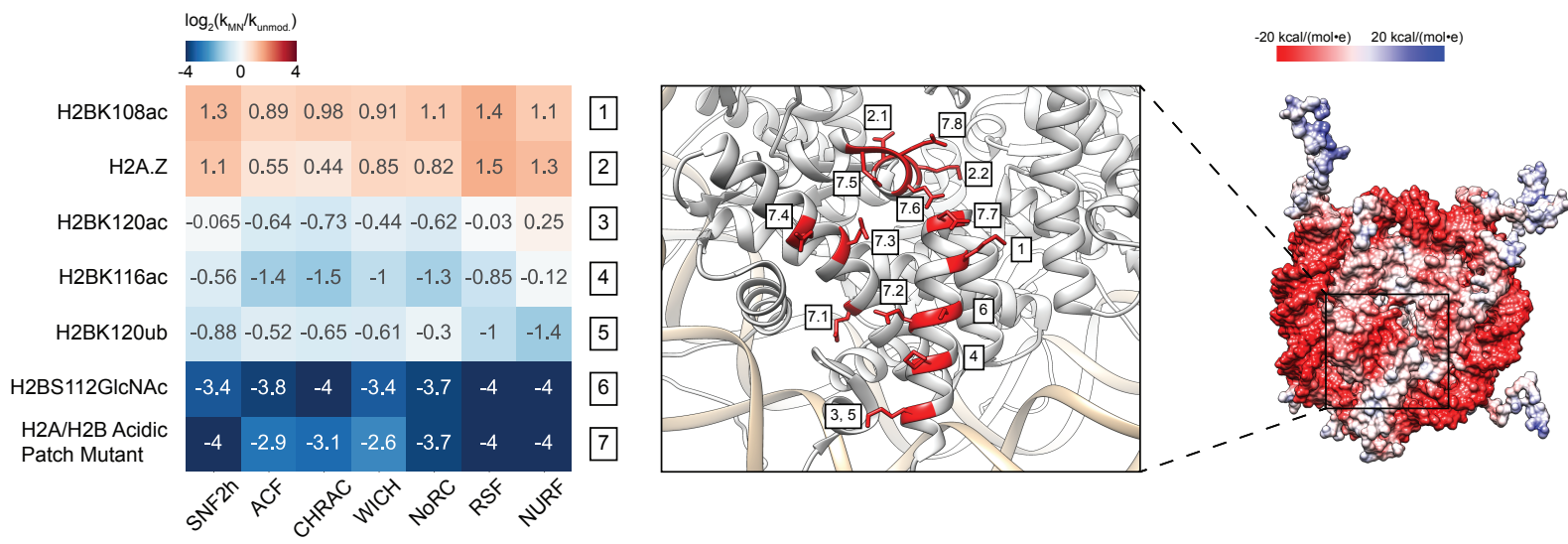
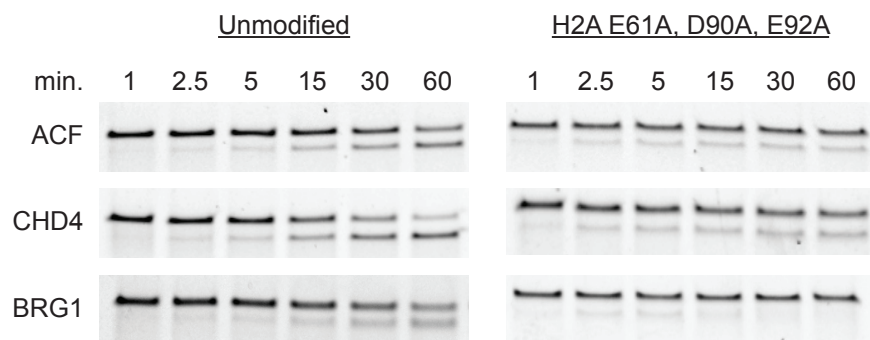
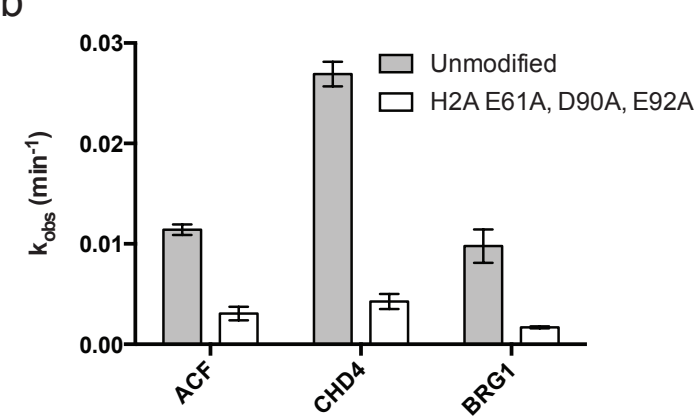
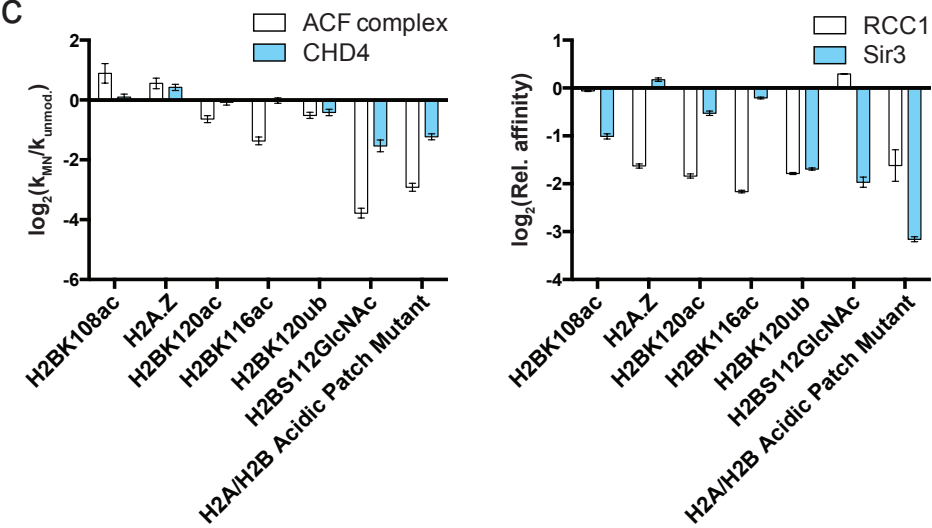


b



c



a**b****c****d**